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Acute and Short-Term Inhalation Toxicity Study of FT Fuel

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14. ABSTRACT
FT (or S-8) jet fuel is a synthetic organic mixture produced using the Fischer-Tropsch (FT) process that transforms natural gas to liquid hydrocarbons. Studies are being conducted to thoroughly evaluate the toxicity of FT jet fuel. First, an acute inhalation toxicity limit test was conducted; FT jet fuel with additives was administered via inhalation exposure to rats once for 4 hours at the maximum specified concentration (2000 mg/m^3). No lethality or adverse clinical signs were demonstrated. In the second study, FT jet fuel, with JP-8 additive package, was administered as an aerosol and vapor mixture via repeated inhalation exposure to rats for 6 hours per day, 5 days per week over 2 weeks at concentrations of 0, 500, 1000 and 2000 mg/m^3. A slight but significant effect on bodyweight was observed in males exposed to the high (2000 mg/m^3) concentration. In the nasal tissues at the intermediate (1000 mg/m^3) and high (2000 mg/m^3) concentrations, minimal to mild olfactory epithelial degeneration was observed, increasing with the higher concentration, and with deeper penetration into the nose. In the lung, foci of inflammatory cell infiltration were observed in all high and some intermediate concentration exposure group animals. FT jet fuel is similar to or less toxic than JP-8 in equivalent tests. In addition, micronucleus induction was tested; FT jet fuel does not induce micronuclei, indicating that the fuel is not genotoxic. A hydrocarbon fingerprint analysis of the aerosol and vapor phase of the delivered test fuel was performed.

15. SUBJECT TERMS
FT, S-8, Fischer-Tropsch, jet fuel, acute, two week, inhalation, toxicity, micronucleus assay, fingerprint analysis

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PREFACE

Funding for this project was provided through the Air Force Research Laboratory, Propulsion Directorate, Fuels Branch (Dr Tim Edwards, AFRL/RZPF) and the Alternative Fuels Certification Office (AFMC 77 AESW/LF, now ASC/WNN). This research was conducted under contracts FA8601-07-P-0448 and FA8601-07-P-0473. The program manager for the contracts was LT Dean Wagner, PhD, USN while he was stationed at the Naval Health Research Center/Environmental Health Effects Laboratory (NHRC/EHEL) Wright-Patterson AFB. The technical manager for the program under which this project was conducted, Fischer Tropsch (FT) Jet Fuel Toxicity Assessment, was Dr David Mattie. The authors acknowledge the following individuals who also served on a review panel for this program and this project: John Hinz (USAFSAM/OEHTH, Brooks City Base, TX); Gunda Reddy, PhD (USACHPPM, Aberdeen Proving Ground, MD); David Steup, PhD (Shell Oil Company, Houston, TX; Chairman, American Petroleum Institute-Toxicology Task Force); and Errol Zeiger, Ph.D., J.D. (Errol Zeiger Consulting, Chapel Hill, NC).

This study was conducted in compliance with the United States Environmental Protection Agency (U.S. EPA) Good Laboratory Practice Standards (40 CFR Part 792), with few noted exceptions.

The study protocols were designed to be in general compliance with the U.S. EPA Office of Prevention, Pesticides and Toxic Substances (OPPTS) Guideline 870. 1300 Acute Inhalation Toxicity (1998) and the Organisation for Economic Co-operation and Development Guideline OECD 412 Repeated Dose Inhalation Toxicity: 28-day or 14-day Study (OECD, 1981).

These animal studies were approved by the Air Force Surgeon General's Human and Animal Research Panel (protocol numbers FWR-2008-0002A and -0004A) and the Hamner Institutes for Health Sciences Animal Care and Use Committee (protocol numbers 07034 and 08002). These studies were conducted in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International, in accordance with the Guide for the Care and Use of Laboratory Animals (NRC, 1996).

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1.0 SUMMARY

FT (or S-8) jet fuel is a synthetic organic mixture produced using the Fischer-Tropsch (FT) process that converts small molecules such as carbon monoxide and hydrogen or methane (natural gas) to liquid hydrocarbons. Two studies were conducted. The first was designed to assess the acute inhalation toxicity of a FT jet fuel with additives when administered via inhalation exposure to rats once for 4 hours at the maximum specified concentration (2000 mg/m^3) according to the U.S. Environmental Protection Agency (EPA) Health Effects Test Guidelines, OPPTS 870.1300 (U.S. EPA, 1998). The average total concentration was 2044 mg/m^3 with an aerosol concentration that averaged 596 mg/m^3. As no lethality or adverse clinical signs were demonstrated, this acute inhalation toxicity study was described as a limit test and no further testing for acute inhalation toxicity was needed.

The second study assessed the potential inhalation toxicity of FT jet fuel, with JP-8 additive package, when administered as an aerosol and vapor mixture via repeated inhalation exposure. Rats were exposed for 6 hours per day, 5 days per week over 2 weeks at concentrations of 0, 500, 1000 and 2000 mg/m^3 according to the Organisation for Economic Co-operation and Development Guideline (OECD) 412 Repeated Dose Inhalation Toxicity: 28-Day or 14-Day study (OECD, 1981). By adding the appropriate control animals, this study also assessed the potential for micronucleus induction. Only one significant clinical sign (nasal discharge) was seen. A slight effect on bodyweight was observed, with males and females exposed to the high (2000 mg/m^3) concentration weighing 11 and 5 percent less than controls, respectively. The decreases were statistically significant for males but not for females. Food consumption differences did not appear to be dose-related. No adverse effects were seen in the trachea, larynx, spleen, adrenals and heart when examined histopathologically. In the nasal tissues of male and female rats at the intermediate (1000 mg/m^3) and high (2000 mg/m^3) concentrations, minimal to mild olfactory epithelial degeneration was observed with increasing concentration, and with deeper penetration into the nose. In the lung, foci of inflammatory cell infiltration were observed in the high concentration group and somewhat in the intermediate group. Hepatocyte hypertrophy, consistent with cytochrome P450 induction, was observed in all male groups and the high concentration female group. This observation was considered to be adaptive. Hyaline droplet accumulation was observed in the kidney cells of all male rats exposed to FT jet fuel.

Toxicity of FT jet fuel to bone marrow cells was not observed in the micronucleus assay. These results indicate that FT jet fuel is not likely to be genotoxic.

A fingerprint analysis of the aerosol and vapor phase of the delivered test chemical mixture was performed which determined the hydrocarbon fingerprint of the FT jet fuel in the chambers by GC/MS. A number of peaks, such as n-octane, n-nonane and n-tetradecane, were qualitatively identified in the FT fuel mixture.

Overall, these studies indicate that FT jet fuel is similar to or less toxic than JP-8. Additional toxicity studies with FT jet fuel, including a 90-day inhalation study and a sensory irritation study, have been performed and are being reported elsewhere.

2.0 INTRODUCTION

FT (or S-8) jet fuel is a synthetic organic mixture produced using the Fischer-Tropsch (FT) process that converts small molecules such as carbon monoxide and hydrogen or methane (natural gas) to liquid hydrocarbons. Synthetic jet fuel is being developed to replace or augment petroleum-derived JP-8 jet fuel for military use by the U.S. Air Force. JP-8 fuel contains a mixture of aliphatic and aromatic hydrocarbons. The FT process for S-8 creates a mixture of aliphatic compounds similar to those found in JP-8, but does not form aromatic chemicals such as benzene and naphthalene compounds (Hemighaus, 2007). This difference of composition between FT and JP-8 fuel points to a potential difference in the toxicity of the two fuels.

During refueling operations, personnel may be exposed to vapors and aerosols of jet fuel primarily by dermal or inhalation exposure. A review of JP-8 jet fuel toxicity concluded that exposure to JP-8 near the permissible exposure limit (PEL) of 350 mg/m^3 was potentially toxic to the immune, respiratory and nervous systems. Consequently, the National Research Council (NRC) proposed a reduction in the PEL for JP-8 to 200 mg/m^3 (NRC, 2003), a value formally adopted by the U.S. Air Force in AFOSH 48-8. The American Conference of Industrial Hygienists (ACGIH) has also set its threshold limit value (TLV) for kerosene and jet fuel at 200 mg/m^3 (ACGIH, 2003).

As a new fuel, very limited toxicity testing of FT jet fuel has been performed. Since inhalation is a major route of exposure for JP-8 jet fuel, the assessment of toxicity of FT jet fuel by inhalation is needed to assess the risk of replacing or augmenting JP-8 by FT fuel. The FT jet fuel to be tested included additives which were those chemicals normally added to JP-8 jet fuel.

The first study was designed to assess the acute inhalation toxicity of a FT jet fuel with additives when administered via inhalation exposure to rats once for a total of four hours at the maximum specified concentration (2000 mg/m^3). The assessment included clinical observations and gross pathology. The U.S. Environmental Protection Agency (EPA) Health Effects Test Guidelines, OPPTS 870.1300 (U.S. EPA, 1998), describes this approach to acute inhalation toxicity as a limit test in which 5 male and 5 female rats are exposed to 2000 mg/m^3 for 4 h. No control group is required. If no lethality is demonstrated, no further testing for acute inhalation toxicity is needed.

The second study was designed to assess the potential inhalation toxicity of FT jet fuel, with JP-8 additive package, when administered as an aerosol and vapor mixture via repeated inhalation exposure to rats for 6 hours per day, 5 days per week for 2 weeks at concentrations of 0, 500, 1000 and 2000 mg/m^3. This study followed the guidelines of the Organisation for Economic Co-operation and Development Guideline (OECD) 412 Repeated Dose Inhalation Toxicity: 28-Day or 14-Day study (OECD, 1981). A separate group of rats were used as controls for assessment of micronucleus induction in order to complete the genotoxicity testing of FT jet fuel. The results of the Reverse Mutation Assay (Ames Test) and Chromosome Aberration Test were reported in Mattie *et al.* (2011). No genotoxicity was found in response to FT jet fuel in either of these *in vitro* assays.

Additionally, in order to better understand any of the potential adverse biological effects the studies may produce, a fingerprint analysis of the aerosol and vapor phase of the delivered test chemical mixture was performed. The goals associated with the fingerprint analysis were (1) to determine the hydrocarbon fingerprint of FT jet fuel by GC/MS for future comparison to aerosolized FT jet fuel samples; (2) to qualitatively identify as many of the peaks present in the FT fuel mixture as possible; and (3) to collect and analyze FT jet fuel during an animal exposure with the intent of analyzing the aerosol phase and the gas phase independent of each other.

3.0 METHODS

The FT jet fuel (S-8 Synthetic Jet Fuel, CAS No. 437986-20-4) was obtained from the manufacturer (Syntroleum Corporation, Tulsa, OK) by the Air Force Research Laboratory Fuels Branch (AFRL/RZPF) at Wright Patterson Air Force Base (WPAFB). An additive package consisting of chemicals normally added to JP-8 jet fuel to prevent fuel icing and corrosion was mixed with the FT jet fuel by AFRL/RZPF. The combination of FT jet fuel with additives was designated POSF 5109 by the Fuels Branch. The FT jet fuel with additives was stored in a well-ventilated area at room temperature. The method of synthesis of the FT jet fuel is maintained by the manufacturer. The jet fuel was not diluted prior to use. The Fuels Branch shipped the FT jet fuel to The Hamner Institutes for Health Sciences to conduct the exposures and analyses for this study. The stability of the jet fuel was tested at the end of the 90-day study, which was conducted shortly after the completion of the two-week study and is being reported elsewhere.

3.1 Animals and Animal Husbandry

The Hamner Institutes for Health Sciences is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). This study complied with the Animal Welfare Act regulations promulgated by the U.S. Department of Agriculture.

3.1.1 Acute Exposure. A total of six male and six female rats (Fischer (CDF®) [F344/DuCrl]) were obtained from Charles River Laboratories, Kingston, NY. Animals were 6 weeks old at time of receipt, with males ranging from 105.1 to 124.0 g and females from 112.3 to 118.6 g. Animals were acclimated to the facility for approximately three weeks. During the acclimation period, animals were individually housed in a stainless steel wire-mesh cage (R-24 cage unit, Lab Products, Inc., Seaford, DE). Animals were assigned a temporary identification number and cage location.

Room conditions were maintained at 22 ± 3°C, 50 ± 20 percent humidity, with a 12 hour light/dark cycle. Animals were fed a certified rodent diet, NIH-07 pellets (Zeigler Brothers, Gardners, PA) and reverse osmosis purified municipal tap water, *ad libitum*, except during exposure, when food was withheld. Certification of feed batch was supplied by the manufacturer. There were no known contaminants in the feed that were expected to interfere with the results of this study. Drinking water analyses were conducted quarterly by an

independent laboratory. There were no known contaminants in the drinking water that were expected to interfere with the results of this study.

Prior to exposure, animals were weighed and 5 males and 5 females were assigned randomized animal numbers using a Provantis™ NT-2000 protocol (Instem Provantis™, Conshohoken, PA) and identified using ear tags (Table 1). Animals were transferred into a similar wire mesh cage in a one cubic meter exposure chamber (H1000, Lab Products, Seaford, DE) just prior to exposure. Animals were exposed for four hours to the aerosol-vapor mixture of FT jet fuel with additives. Approximately one hour after the exposure ended, animals were removed from the exposure chamber and placed back into their housing cage.

Following the exposure, animals were maintained in wire caging for 14 days, per guideline. All animals survived through the post-exposure hold period. On Study Day 14, animals were euthanized and necropsied. Animals euthanized were deeply anesthetized with sodium pentobarbital (intraperitoneal injection, approximately 30 mg/kg) and exsanguinated by transection of the abdominal aorta. The necropsy included examination of the external surface and all orifices; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass. The pathology observations were conducted by Dr. Gabrielle A. Willson, B.V.M.S., MRCVS, F.R.C. Path (Experimental Pathology Laboratories, Inc. (EPL), Research Triangle Park, NC). As no lesions or other unusual findings were observed, no further examinations were made and no tissues were taken.

Table 1. Study Designs

Acute Exposure Group	Exposure Level mg/m^3	Number of Animals	
		Males	Females
High	2000	5	5
Quality Control	NA	1	1
Total		6	6

Ten-Day Exposure Group	Exposure Level mg/m^3	Number of Animals	
		Males	Females
Control	0	5	5
Low	500	5	5
Intermediate	1000	5	5
High	2000	5	5
Micronuclei Negative Control	NA	5	5
Micronuclei Positive Control	NA	5	5
Quality Control	NA	2	2
Total		32	32

3.1.2 Ten-Day Exposure. A total of 32 male and 32 female rats (Fischer (CDF®) [F344/DuCrl]) were obtained from Charles River Laboratories. Animals were six weeks of age and appeared to be in good health. Male weights ranged from 104.1 to 139.9 g and female weights ranged from 92.7 to 116.1 g the day after receipt. Animals were acclimated to the facility for approximately two weeks. During the acclimation period, animals were individually housed in stainless steel wire-mesh cages (R-24 cage units). At the start of exposures, male weights ranged from 152.8 to 187.1 g and female weights ranged from 116.3 to 141.0 g. Weight gain after receipt at the testing facility indicated that animals maintained good health during the acclimation period.

Room conditions were maintained between 20 and 24°C, 30 to 70 percent humidity, with a 12 hour light/dark cycle. Animals were fed a certified rodent diet, NIH-07 pellets (Zeigler Brothers,

Gardners, PA), and reverse osmosis purified municipal tap water, *ad libitum*, except during exposure, when food was withheld.

Prior to exposure, 30 males and 30 females were selected for study. Animals were weighed and assigned randomized animal numbers using a Provantis™ NT-2000 protocol. Each animal was identified with a stainless steel ear tag stamped with the animal number. For each exposure group, five male and five female Fischer-344 rats were used (Table 1). Animals were housed in wire mesh caging units; the units were transferred into one cubic meter exposure chambers (H1000) just prior to exposure. Animals were exposed for six hours per day, five days per week, for two weeks to the aerosol-vapor mixture of jet fuel. Approximately one-half to one hour after each exposure period, caging units were transferred to similarly prepared one cubic meter chambers for housing during non-exposure periods. Control animal caging units remained in their original exposure chamber.

During the weekend between exposure weeks, food consumption was measured. Following the exposure on Friday, animals in the exposure groups were transferred to individual polycarbonate caging. Food was added to the stainless steel wire cage lid and weighed. On the following Monday morning, animals were transferred back to the exposure chambers. The stainless steel wire cage lid was weighed to determine the amount of food consumed.

Following the last exposure, animals were weighed, euthanized by an overdose of sodium pentobarbital, exsanguinated by transection of the abdominal aorta; and then necropsied. The necropsy included examination of the external surface and all orifices; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass. The pathology observations were conducted by Dr. Gabrielle A. Willson of EPL. Wet weights of the lungs, liver, kidneys, adrenals and testes were obtained after dissection from the exposure animals (but not from the micronucleus control animals).

The respiratory tract tissues of all study animals (except for animals designated as the micronucleus (MN) assay positive and negative controls) and other tissues were fixed and stained for microscopic examination using appropriate methods. Histological slides were prepared at EPL and microscopic examinations were performed by Dr. Gabrielle A. Willson of EPL. These tissues included the trachea, larynx, lungs (two sections), liver (two sections), kidney (right and left), spleen, adrenals (right and left), heart, and nasal cavity (four sections). Tissues from the high concentration and control groups were initially examined histologically. If treatment-related changes were detected, additional tissues from the low and intermediate concentration groups were examined.

3.1.3 Micronucleus Assay. Bone marrow samples from the femurs of exposed animals were extracted for a micronuclei induction assay. A bone marrow smear was prepared, stained and erythrocytes examined for polychromatic erythrocytes (PCEs), normochromatic erythrocytes (NCEs) and micronucleated PCEs or micronuclei, round bodies in the cytoplasm with a diameter of 1/20 to 1/5 of an erythrocyte. Micronuclei stain intensively, similar to the staining of the main nuclei in nucleated cells, and are an indication of genotoxicity (Heddle *et al.*, 1983).

To assure the responsiveness of the test subjects to micronuclei induction, cyclophosphamide (CP), a well known inducer of micronuclei, was dissolved in saline and injected intraperitoneally (IP) to a positive control group of rats (5 males and 5 females, Table 1) 24 hours before the euthanization of inhalation exposed animals. A negative control group of rats (five males and five females) received saline IP as a vehicle control. The negative and positive control animals were observed for general health, but did not undergo a full necropsy or have tissues taken except for bone marrow.

3.2 Exposure System

Air for the exposure chamber was pulled by a fan through a high efficiency (95 percent) particulate air (HEPA) filter and a charcoal filter. The temperature and humidity were adjusted as required prior to distribution to the H1000 exposure chamber. Air flow was measured by monitoring the pressure drop across an orifice plate at the inlet to the chamber. Air flow was calibrated using an in-line mass flow meter (Sierra Instruments, Inc., Monterey, CA). The temperature and relative humidity in the chamber was measured by using a humidity temperature transmitter (Hygromer 200 Series, Rotronic AG, Bassersdorf, Switzerland). The temperature transmitter was calibrated by comparison with a certified thermometer, and humidity was calibrated by comparison with saturated salt solutions.

3.3 Generation System

Aerosol and vapor of FT jet fuel with additives were generated by pumping the liquid jet fuel into an air atomizing nozzle (Model SUJ1A with fluid cap 1650 and air cap 64, Spraying Systems Co., Wheaton, IL). A liquid metering pump (Model QVG50-Q2 with 3/8 inch piston and stainless steel pump head, Fluid Metering, Inc., Syosset, NY) pumped liquid jet fuel from a glass bottle reservoir to the nozzle. Compressed instrument air at approximately 50 psi was supplied to the nozzle. The nozzle assembly was housed in a stainless steel sanitary tee fitting. The spray was directed into a custom-made mixing tube, consisting of a large glass tube (165 mm OD (6.5 in) by 610 mm (24 in) in length) that tapered at both ends to smaller glass tubes (63.5 mm OD (2.5 in) by 25 mm (1 in) long), with an overall length of approximately 864 mm (34 in) (Figure 1).

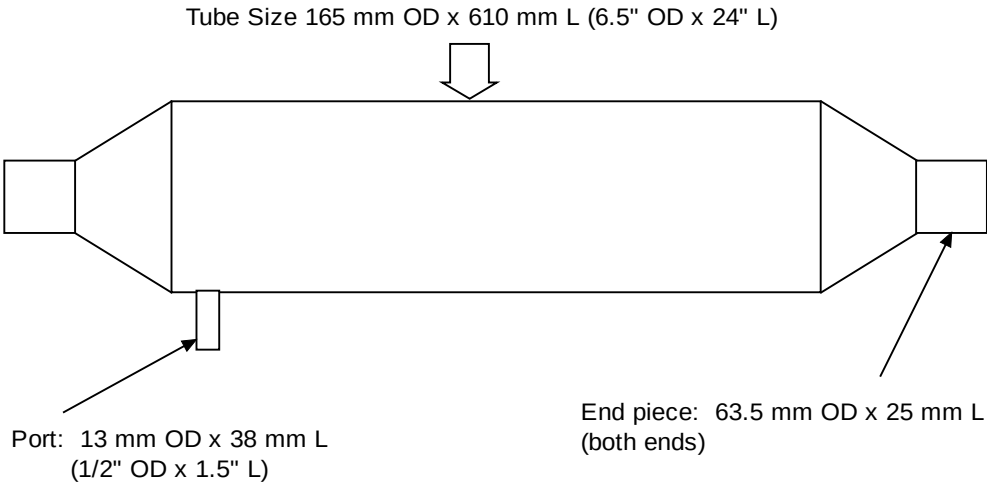


Figure 1. Glass Mixing Tube
Custom-made glass mixing tube for FT jet fuel generation system. Overall length is approximately 864 mm.

The mixing tube dimensions were designed to contain the jet plume for mixing with the chamber air, while minimizing impaction or intersection of the jet plume with the walls of the tube. Loss of aerosol to the walls was not observed. The total flow of the chamber passed through the glass tube, carrying the generated jet fuel mixture into the exposure chamber (Figures 2 and 3). The pump (FMI, Fluid Metering, Inc., Syosset, NY) flow rate was calibrated by pumping liquid into a graduated cylinder, and measuring the time to accumulate a specified volume. The FMI pump produced a pulsatile liquid flow and ultimately generated a pulsing aerosol concentration into the chamber air flow. The pulsations were of the order of seconds, so the mixing with the chamber air flow and subsequent residence time in the exposure chamber dampened the pulsations to the point where the aerosol concentration was stable.

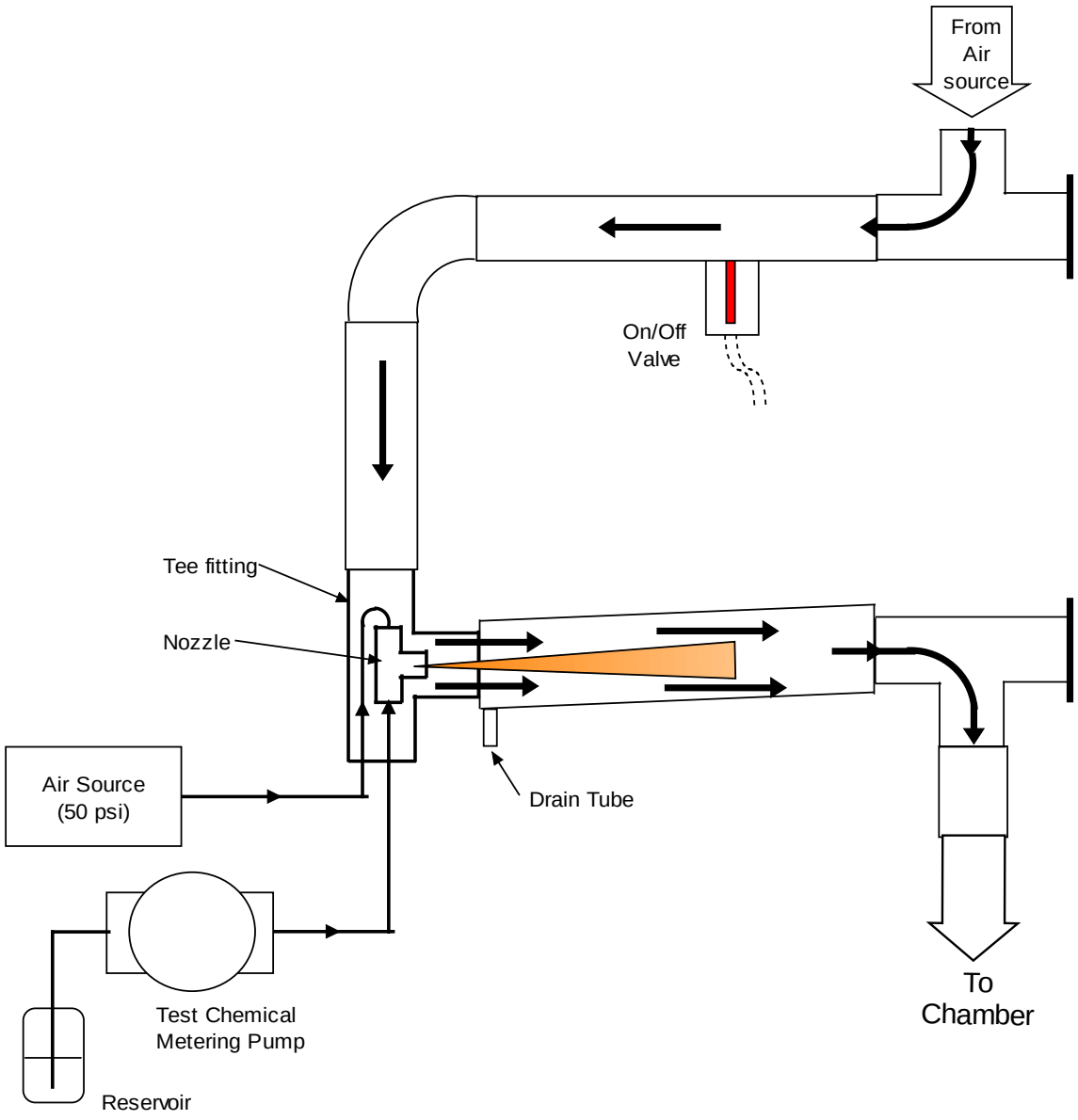


Figure 2. Jet Fuel Generation System

Diagram of FT jet fuel generation system showing the spray nozzle, glass mixing tube and associated parts. For this study, the system was operated with the On/Off valve completely open with all of the air flow passing through the glass mixing tube.

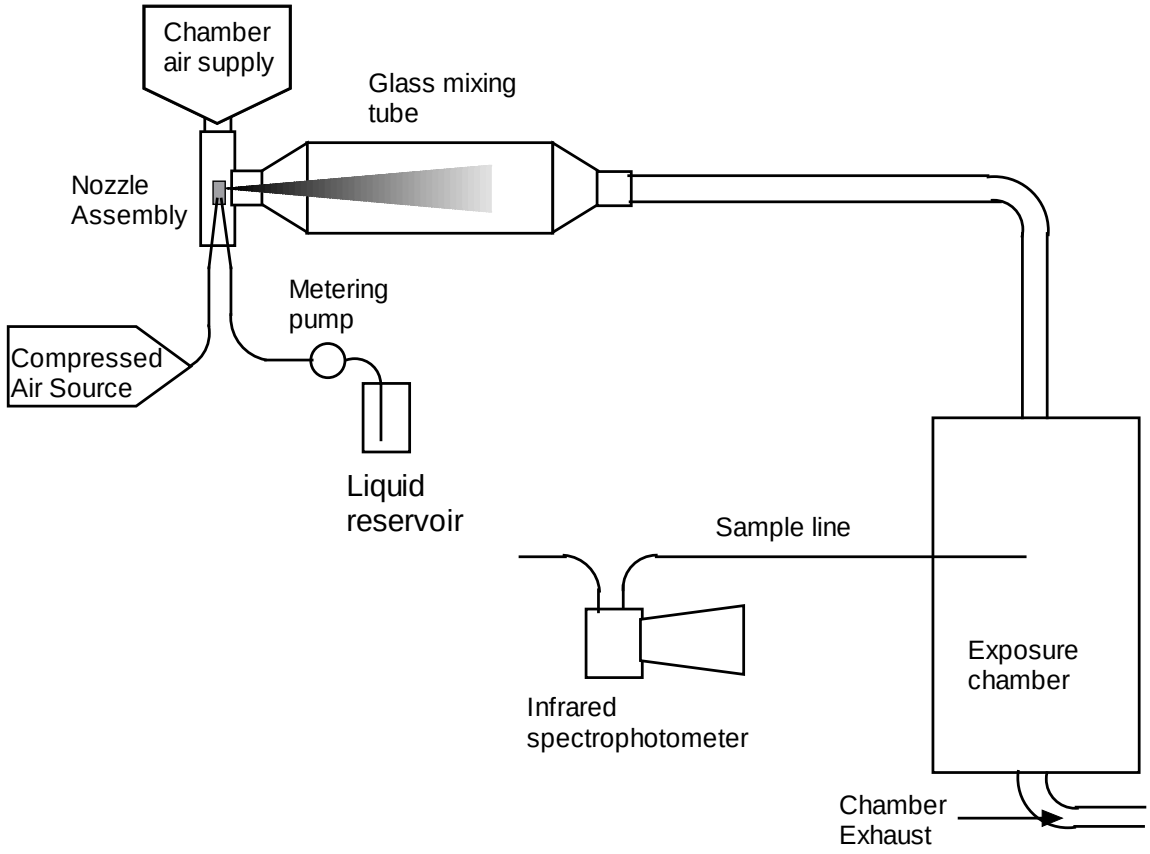


Figure 3. Schematic of FT Jet Fuel Generation, Exposure and Sampling System

3.4 Infrared Spectrometer Concentration Measurement

An infrared (IR) spectrophotometer (MIRAN 1A, Foxboro Co., South Norwalk, CT) was used to monitor the concentration of FT jet fuel with additives in the chamber. The spectrophotometer settings are listed in Table 2. The sensing cell of the IR spectrophotometer was warmed to approximately 50°C by a heat tape connected to a variable transformer and covered by a sheet of neoprene rubber for insulation. The temperature was monitored by a thermometer. A sample of the chamber atmosphere was pulled through the IR spectrophotometer (Figure 3). Due to the heat of the sensing cell, the aerosol droplets evaporated, causing the measured concentration to include the existing vapor and the aerosol concentration. A chart recorder was used to continuously record the electrical output of the IR spectrophotometer.

The infrared spectrophotometer was calibrated using a closed loop method. A stainless steel diaphragm pump was connected to the inlet and outlet of the infrared spectrophotometer with specified lengths of tubing with a fitting containing a septum on the inlet side to produce a closed system with a specified volume. Clean air was circulated and a scan of response as a function of wavelength was conducted. A small sample of FT jet fuel with additives was injected and a scan superimposed over the clean air scan to identify a representative peak. The infrared wavelength was set at the identified wavelength. Clean air was readmitted into the infrared

spectrophotometer, and then jet fuel was injected in a series of volumes to produce a set of increasing concentrations of jet fuel. A calibration curve of spectrophotometer response as a function of jet fuel concentration is shown in Figure 4. Nominal concentration was calculated from the air flow rate through the chamber and the FMI pump rate.

Table 2. Infrared Spectrophotometer (MIRAN) Parameters

Target Concentration (mg/m^3)	2000
Serial No.	4211
Wavelength (µm)	3.52
Path length (m)	2.25
Slit (mm)	2
Range	1A
Response Time (sec)	4
Coarse zero	1x
Calibration Curve y = concentration (mg/m^3) x = output voltage	$y = 3298.5x^2 + 3488.4x - 0.6998$
Correlation Coefficient	1.0
Calibration Range (mg/m^3)	0 to 3324.5
Estimated Limit of Detection	16.8

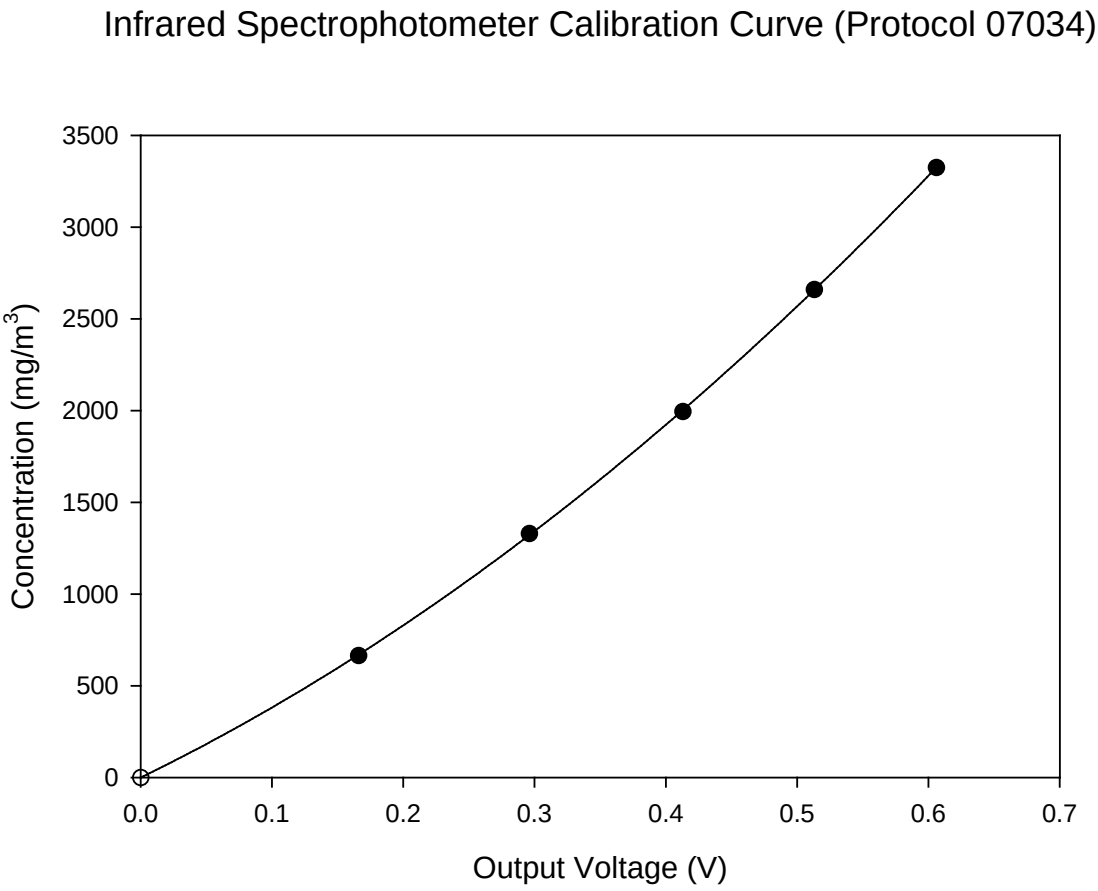


Figure 4. Calibration Curve for Infrared Spectrophotometer
The concentration of jet fuel (mg/m^3) as a function of the instrument output voltage.

3.5 Gas Chromatograph Concentration Measurement

Initially, a gas chromatograph (GC, Model 5890, Hewlett Packard, San Jose, CA) was set up to measure the concentration within the chamber. A sample of the exposure atmosphere was continuously pulled through a sampling line from the chamber to the GC. A small sample would then be periodically measured by the GC. The GC was calibrated by sampling from Tedlar bags containing specified concentrations of FT jet fuel with additives. Calibration bags were prepared by filling Tedlar bags with known volumes of air and injecting specified volumes of FT jet fuel with additives.

However, it was determined that the GC was not accurately measuring the bag standards. This could be attributed to the loss of some jet fuel components due to adsorption on the walls of the bag. If the bag walls were heated by a heat gun, the relative concentration reported by the GC would rise. Attempts were made to calibrate the GC by carefully heating the bags during the calibration procedure, but it was still difficult to get stable readings. Because of the uncertainty of the GC calibrations, the infrared spectrophotometer was used to characterize the atmosphere. Chamber distribution measurements are found in Table 3.

3.6 Light Scattering Aerosol Measurement

A light scattering real-time aerosol sampler (RAM-S, MIE, Inc., Billerica, MA) was used to measure aerosol concentration. A sample of the chamber atmosphere was pulled through the instrument. Light scatter from the aerosol was detected and the detector signal recorded on a chart recorder. Dilution air was added to the sample line in order to reduce the concentration to avoid overloading the instrument detector. Over the course of the trial runs and actual exposure, it was observed that readings from the instrument, while providing a continuous measure of the aerosol concentration, did not provide readings that could be standardized sufficiently to permit the use of this instrument to measure aerosols in the chamber.

3.7 Chamber Distribution: Ten-Day Study

The uniformity of distribution within the exposure chamber was checked by measuring the concentration at four different locations within the chamber and from the home port (primary sampling port) (Table 3). Chamber distribution measurements were conducted using the infrared spectrophotometer and it was determined that the variability in chamber concentration was less than four percent, indicating that the distribution of the test compound within the chamber was uniform.

Table 3. Chamber Distribution Measurements

Sample Position[1]	Chamber 405A Temporal or Within Port (WP) Concentration (mg/m^3)	Chamber 405A Total Port (TP) Concentration (mg/m^3)
Center[2]	1753.4	
1		1845.8
2		1940.8
Center	1783.2	
3		1904.1
4		1873.6
Center	1819.2	
		1785.3[3]
Average	1785.3	1869.9
Std. Dev.	32.9	59.05
N	3	5
TPCV [4]	3.2%	
WPCV [5]	1.8%	
BPCV [6]	2.6%	

Notes: Adapted from Cheng and Moss (1995); [1]Sample positions: 4 corners of rectangular cage unit and chamber center; [2]Center sample position is the standard position for monitoring the chamber; [3]Average of three samples taken at the center position during the distribution test; [4]TPCV = Total Port Coefficient of Variation: (standard deviation (SD) TP/Average TP) * 100; [5]WPCV = Within Port Coefficient of Variation: (SD WP/Average WP) * 100; [6]BPCV = Between Port Coefficient of Variation: $\sqrt{[(TPCV)^2 - (WPCV)^2]}$

3.8 Aerosol Concentration and Particle Size Distribution

As the FT jet fuel with additives is a mixture of organic compounds of varying volatility, equilibrium exists between the jet fuel aerosol droplets and vapor in the exposure chamber that may be altered when the aerosol is collected on the filter. The sampling of an atmosphere of droplets and vapors has been an area of research (Volckens *et al.*, 1999). In that study, various methods, including gravimetric filter samples, for measuring aerosol concentration were evaluated. The results indicated that at higher mist concentrations, the methods provided similar values. Concentrations used in this study were higher than in the aforementioned study, indicating that a gravimetric filter should provide adequate measurement of aerosol concentration. Also, preliminary results from trial runs showed similar aerosol/vapor ratios to other jet fuel studies.

Aerosol concentration was determined by taking a gravimetric filter sample from the chamber. A weighed filter was connected to a sample port on the chamber. After pulling a sample of the

atmosphere through the filter at a known flow rate and time, the filter was reweighed. The aerosol concentration was calculated from the mass of the jet fuel collected on the filter and the volume of atmosphere pulled through the filter.

Particle size distribution measurement was conducted using an aerodynamic particle sizer (APS, Model 3321, TSI, Inc., Shoreview, MN). The instrument was connected to a sample port on the chamber. Dilution air was added in order to keep the aerosol concentration out of overload conditions.

3.9 Statistics

Basic summary statistics, including means and standard deviations, were compiled for most exposure and in-life data. Additional tests of significance for in-life data were computed by the Instem Provantis™ 8 system (Instem Provantis™, Conshohoken, PA).

3.10 Stability and Fingerprint Analyses

Following the exposures, a sample was taken from the jet fuel drum at the Hamner Institutes and was sent to the Fuels Branch at WPAFB. A sample was then taken of the stock at WPAFB, from which The Hamner's jet fuel originally came. Gas chromatographic analysis for stability was conducted at WPAFB; this analysis was not conducted under U.S. Environmental Protection Agency (EPA) Good Laboratory Practices (GLP).

The FT jet fuel with additives was characterized as part of a separate project (not conducted under GLP) by the Analytical Chemistry Services Group at The Hamner Institutes. Aerosol and vapor phase FT jet fuel samples were collected from the exposure chambers during high (2000 mg/m^3), middle (1000 or 700 mg/m^3) and low (500 or 200 mg/m^3) FT fuel exposures for the purpose of qualitatively comparing the various samples using gas chromatography/mass spectroscopy (GC/MS) analysis.

4.0 RESULTS

4.1 Exposure Period

4.1.1 Acute Exposure. An acute four-hour exposure was started when the compressed air and the jet fuel flow were applied to the nozzle and ended when air and fuel flow to the nozzle stopped (Cheng and Moss, 1995). The concentration in the chamber began to increase immediately, as observed on the infrared spectrophotometer chart recording. At the end of the four-hour exposure period, the compressed air and fuel flow to the nozzle were shut off. The aerosol concentration dropped as expected (data not shown). The overall vapor concentration, however, took significantly longer than expected to decrease.

Prior to this acute exposure to aerosol and vapor of FT jet fuel with additives, an inadvertent exposure had occurred. The animals were exposed to some remnants of jet fuel vapors a week prior to the exposure. A trial run was conducted with the acute exposure scheduled for the next day. In preparation for the planned exposure the next day, the test animals were placed into the chamber approximately one hour following the end of a trial run. Results of the trial run indicated that issues with the gas chromatography method (as discussed above) were unresolved, and a decision was made to delay the exposure for a week. The following morning, animals were removed from the chamber and placed back into their animal housing room. However, results from the trial run indicated that the FT jet fuel with additives was not cleared from the chamber as rapidly as expected (based on flow through the chamber and verified in subsequent trial runs). While in the chamber after the trial run, the animals were exposed to the jet fuel vapors left over from the trial run.

As the animals were exposed to remnants of the jet fuel, a decision whether to order a new batch of animals with an additional delay of the exposure, or continue with the existing group of animals was made in favor of continuing with the existing group. The acute exposure was conducted the following week. As the primary objective for this acute exposure was to determine if animals could tolerate the exposure concentration at the maximum level mandated by the U.S. EPA guidelines, any acute or short term clinically observable effects would most likely be attributable to the high level concentration of aerosol and vapor, and not to the previous, much lower concentration of vapor only, and if no effects were observed, then neither exposure would have had an effect.

4.1.2 Ten-Day Exposure. As with the acute study, the exposure period started when the compressed air and the jet fuel flow were applied to the nozzle. The concentration in the chamber began to increase immediately, as observed on the infrared spectrophotometer chart recording. At the end of the exposure period, the compressed air and fuel flow to the nozzle were shut off. The aerosol concentration dropped as expected. As before, the vapor concentration took significantly longer to clear the chamber. In order to avoid exposing the animals to this long tail of hydrocarbons, animals were moved from the exposure chamber to the respective housing chamber by moving the entire wire mesh rack from one chamber to the other. Control animals were exposed and housed within the same chamber. However, the rack was pulled out and pushed back into the chamber to simulate the rack movements.

4.2 Exposure Conditions

4.2.1 Acute Exposure. Over the course of the exposure, concentration, temperature, humidity, air flow and static pressure readings were manually recorded (Table 4). The average temperature (21.9 ± 0.2°C), humidity (49.3 ± 2.3 percent) and air flow (225.2 ± 1.1 L/minute) remained at target levels, and did not deviate outside of prescribed ranges. The overall concentration was 2044.0 ± 20.7 mg/m^3. Nominal concentration, based on the liquid pump flow rate and the chamber air flow, was 1957 mg/m^3. The analytical (measured) concentration to nominal

(theoretical) concentration ratio was 1.04. A ratio less than one could be indicative of inefficiency in generation or loss of test material before reaching the exposure chamber. A ratio greater than one indicates that test material is being created, a physical paradox, and is likely attributed to variability in the measurements or calibration of the various parameters involved, such as the jet fuel pump rate, the chamber inlet air flow, or the infrared spectrophotometer.

Table 4. Acute Exposure Conditions

Elapsed Time (min)	Overall Concentration (mg/m^3)	Temperature (°F)	Humidity (% RH)	Air Flow (L/min)	Static Pressure (in H_2O)	Pump Flow (mL/min)
0	0	71.1	46.0	224.6	-0.123	0.610
7	867.6					
15	1676.7	71.1	47.3	224.6	-0.140	
35	2052.5					
43	2071.4	71.3	47.3	224.6	-0.140	0.591
68	2071.4	71.3	49.3	224.9	-0.224	
100	2021.3	71.8	53.0	224.6	-0.239	0.575
122	2033.8	71.8	51.6	227.5	-0.224	
150	2058.8	71.8	49.0	224.9	-0.176	
190	2021.3	71.8	48.6	226.6	-0.199	0.575
232	2021.3	71.8	51.4	224.9	-0.171	
Average	2044.0*	71.5 (21.9°C)	49.3	225.2	-0.182	0.588
SD	22.2*	0.3 (0.2°C)	2.3	1.1	0.042	0.016
CV (%)	1.0	0.4	4.7	0.5	23.1	2.7

*Concentration average and standard deviation includes values after 15 minutes of elapsed time (Cheng and Moss, 1995); CV = coefficient of variation; min = minutes; SD = standard deviation

The aerosol mass concentration was measured using gravimetric filters. Three filter samples were taken during the course of the exposure (Table 5). The average aerosol concentration was 596.1 mg/m^3. The aerosol comprised 29 percent of the total FT jet fuel concentration in the chamber.

Table 5. Acute Exposure Aerosol Mass Concentration

Sample Start Time (min)	Sample time (min)	Mass Concentration (mg/m^3)
45	25	645.9
90	32	570.3
208	20	572.2
	Average	596.1
	SD	43.1
Fraction of total concentration		29%

Note: min = minutes; SD = standard deviation

A series of particle size distribution measurements were made over the course of the exposure (Table 6). The aerosol particle size, as measured by the APS, gave a mass median aerodynamic diameter (MMAD) of 2.7 µm with a geometric standard deviation (GSD) of 1.6. The aerosol was respirable, and well within the target range of 1 to 4 µm MMAD of the guidelines.

Table 6. Acute Exposure Aerosol Particle Size Distribution Measurements

Elapsed Time (min)	Geometric Mean (μm)	GSD
33	1.26	1.65
44	1.30	1.64
58	1.30	1.64
76	1.32	1.64
93	1.32	1.64
114	1.32	1.64
158	1.32	1.64
169	1.32	1.64
211	1.33	1.64
222	1.32	1.64
Average	1.31	1.64
SD	0.02	0.00
MMAD	2.7	
GSD	1.6	

Notes: GSD = geometric standard deviation; min = minutes; MMAD = mass median aerodynamic diameter; SD=standard deviation; MMAD was calculated from the geometric mean diameter based on the following calculation: MMAD = Geometric Mean * (exp 3* $\ln(GSD)^2$) (Hinds, 1999)

4.2.2 Ten-Day Exposure. Over the course of the exposures, concentration, temperature, humidity, air flow and static pressure readings were recorded (Table 7). The average temperature, humidity and air flow remained at target levels, and did not deviate outside of prescribed ranges. The average total concentrations for the ten-day exposures were 0.5 ± 1.1, 497 ± 8, 999 ± 20, and 1958 ± 42 mg/m^3 for the 0, 500, 1000, and 2000 mg/m^3 chambers, respectively. Nominal concentrations, based on the liquid pump flow rate and the chamber air flow were 478 ± 6, 953 ± 17, and 1938 ± 44 mg/m^3, giving analytical to nominal concentration ratios of 1.04, 1.05, and 1.01, respectively. A ratio greater than one indicated that test material was being created, a physical paradox, and was attributed to variability in the measurements or calibration of the various parameters involved, such as the jet fuel pump rate, the chamber inlet air flow, or the infrared spectrophotometer.

Table 7. Ten-Day Exposure Conditions

	Target Concentration	0 (mg/m^3)	500 (mg/m^3)	1000 (mg/m^3)	2000 (mg/m^3)
1-m^3 Temperature (°F)	Mean of daily means	**71.2**	**70.8**	**71.1**	**72.4**
	SD	0.3	0.2	0.3	0.2
	Maximum daily mean	71.5	71.1	71.4	72.7
	Minimum daily mean	70.6	70.3	70.6	72.0
	# of Days	10	10	10	10
1-m^3 Relative Humidity (%)	Mean of daily means	**54**	**43**	**45**	**51**
	SD	2	1	1	1
	Maximum daily mean	56	45	47	52
	Minimum daily mean	52	41	44	49
	# of Days	10	10	10	10
1-m^3 Air Flow (L/min)	Mean of daily means	**225**	**224**	**225**	**225**
	SD	1	1	2	1
	Maximum daily mean	226	227	227	227
	Minimum daily mean	224	223	221	223
	# of Days	10	10	10	10
Actual Chamber Static Pressure (in H_20)	Mean of daily means	**0.216**	**-0.142**	**-0.381**	**-0.345**
	SD	0.079	0.014	0.068	0.077
	Maximum daily mean	0.273	-0.116	-0.295	-0.241
	Minimum daily mean	0.015	-0.158	-0.486	-0.456
	# of Days	10	10	10	10
Actual Chamber Concentration (mg/m^3)	Mean of daily means	**0.5**	**497.0**	**999.0**	**1958.1**
	SD	1.1	8.4	19.9	41.8
	Maximum daily mean	2.9	507.7	1019.1	2006.0
	Minimum daily mean	0	483.7	947.3	1855.4
	# of Days	10	10	10	10
Nominal Chamber Concentration (mg/m^3)	Mean of daily means	**N/A**	**477.9**	**953.2**	**1938.1**
	SD	N/A	6.3	16.9	43.6
	Maximum daily mean	N/A	487.8	976.1	2019.8
	Minimum daily mean	N/A	470.4	934.8	1880.1
	# of Days	N/A	10	10	10
Mass Concentration (mg/m^3)	Mean	0.27	58.2	141.1	580.6
	SD	0.20	9.4	8.9	24.8
	Aerosol Fraction	NA	0.12	0.14	0.30
Particle Size Distribution	MMAD (µm)	1.00	1.04	1.32	2.66
	GSD	1.38	1.35	1.45	1.65

Notes: GSD = geometric standard deviation; min = minutes; MMAD = mass median aerodynamic diameter; SD = standard deviation

The aerosol mass concentration was measured using gravimetric filters. Filter samples were taken during exposures at the beginning and end of each week over the course of the study, for a total of four samples from each chamber. The average aerosol concentrations were 0.27 ± 0.2, 58.2 ± 9.4, 141.1 ± 8.9, and 580.6 ± 24.8 mg/m^3 (Table 8). Mass collected on the gravimetric filter at 0.27 mg/m^3 in the control chamber either came from the supply air (filtered through 95 percent HEPA filters) or from animals contributing dander, fur, food, or other particles into the atmosphere. The concentration of particles from animals was more than an order of magnitude lower than the lowest aerosol concentration in the exposure chambers, and was not a significant component of the aerosol in the jet fuel exposure chambers. In the exposure chambers, the aerosol comprised 12 percent of the total concentration for the low concentration chamber, and increased to 14 and 30 percent of the total jet fuel concentration in the intermediate and high concentration chambers, respectively. Thus, as the total FT jet fuel concentration increased, the fraction of the total that existed as aerosol droplets also increased.

An aerodynamic particle sizer was used to measure the particle size distribution. Measurements were made by sampling each chamber twice a week, equalling four measurements over the course of the exposure. The average mass median aerodynamic diameter and geometric standard deviation (MMAD (GSD)) of the aerosols were calculated as 1.0 (1.4), 1.0 (1.4), 1.3 (1.5) and 2.7 (1.7) µm for the control, low, intermediate and high concentration chambers, respectively (Table 8). Aerosols with particle size distributions between 1 and 4 µm are generally considered as respirable by rodents.

Table 8. Ten-Day Aerosol Mass Concentration and Particle Size Distribution

Target Exposure Level	Mass Concentration (mg/m^3)	Aerosol Fraction aerosol mass/ total mass	Particle Size Distribution MMAD (GSD) (µm)
0	0.27 ± 0.20	NA	1.00 (1.38)
500	58.2 ± 9.4	0.12	1.04 (1.35)
1000	141.1 ± 8.9	0.14	1.32 (1.45)
2000	580.6 ± 24.8	0.30	2.66 (1.65)

Notes: MMAD = mass median aerodynamic diameter; GSD = geometric standard deviation

4.3 Animal Body Weights

Appendix A contains tabular data from the figures contained in Section 4.3.

4.3.1. Acute Exposure. Animal group mean body weights showed a steady increase from arrival at the facility over the course of the study (Figure 5). A slowing of the growth curve was seen in the period immediately following the exposure (Study Day 0 to Study Day 7), with a

subsequent increase in the growth curve from Study Day 7 through 12. The body weight gain was calculated as the difference in weight divided by the number of days between weighing intervals. Figure 6 shows a decrease in the daily weight gain for both males and females after the animals arrived at the facility (from Study Day -21 to Study Day -7), perhaps due to acclimation to the facility and to wire mesh caging. The body weight gain appeared to level out for the males, and increased for the females for the period from Study Day -7 to Study Day 0. After the exposure at Study Day 0, male daily body weight gain slowed when measured on Study Day 7, and then increased in the following interval (Figure 6). Female body weight gain showed a similar trend, slowing after the exposure and accelerating the following week (Figure 6). There was no comparable control group (a control group is not required for a limit study) so a general change in environment (movement to the exposure chamber) cannot be ruled out as causing the decrease in weight gain. However, the change in male and female body weight gain appeared to be correlated with the exposure to the test material.

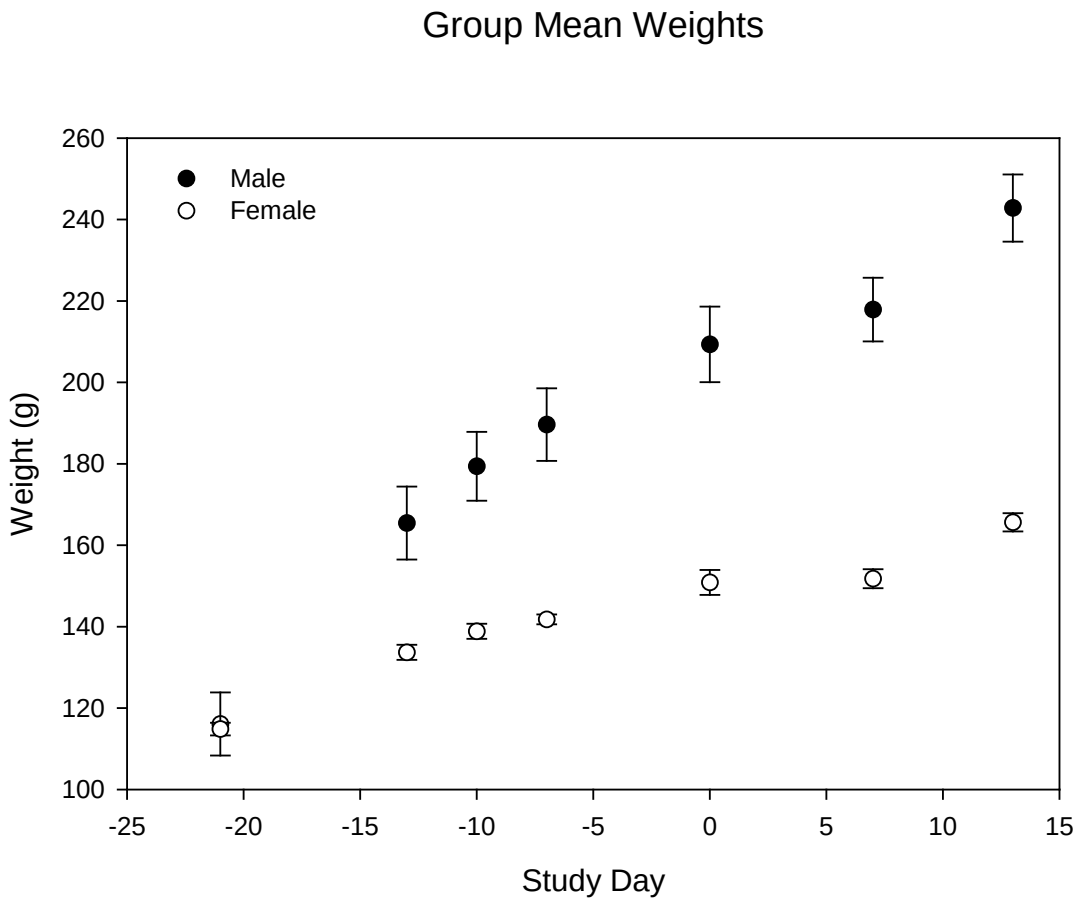


Figure 5. Group Average Weights for Males and Females
Group average weights and standard deviation error bars are plotted as a function of study day. Study Day 0 is the day of exposure.

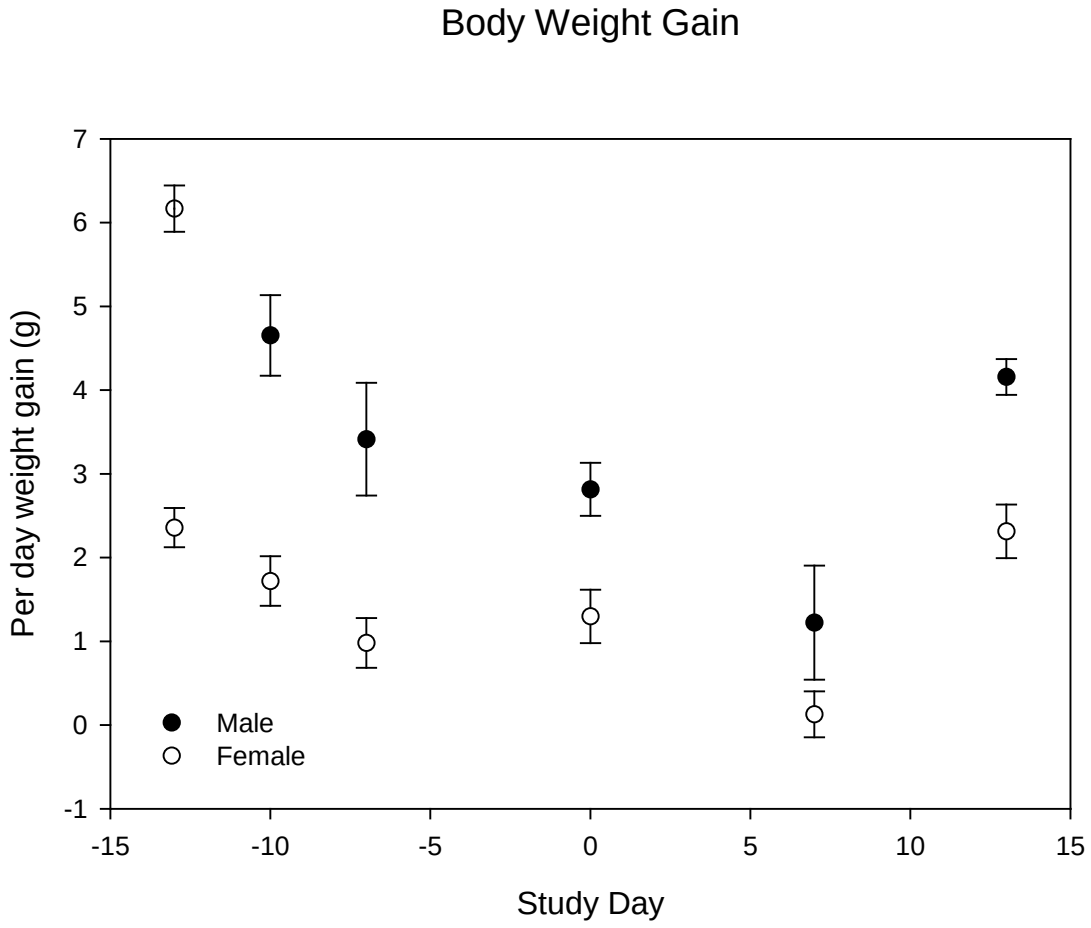


Figure 6. Average Body Weight Gain for Males and Females
The average daily weight gain is plotted at the end of the specified period.

4.3.2 Ten-Day Exposure. Animals gained weight over the course of the study (Figure 7). For both the females and males, the average group weight increased through Study Day 1, when the animals were weighed prior to the start of exposures. The average body weight of the males in the control, low (500 mg/m^3) and intermediate (1000 mg/m^3) exposure groups continually increased, though the rate of weight gain appeared to slow after the start of exposures (following Study Day 1). For males in the high (2000 mg/m^3) exposure concentration group, the average body weight decreased between the start of exposures and Study Day 5, increased over the weekend when no exposures were being conducted, and then decreased again during the second week of the study as seen at the terminal necropsy on Study Day 12 (Figure 7a). At necropsy, the high and intermediate concentration male average body weights were 11 and 5 percent lower, respectively, than controls. The average body weight of the females in all exposure concentration groups showed some decreases between Study Day 8 and Study Day 12 at the terminal necropsy. The average female body weight of the high exposure concentration groups showed a similar pattern as the high concentration male group (Figure 7b). At necropsy, the high concentration female average body weight was five percent lower than controls. A statistical test (Provantis™ "Default Decision Tree") performed on the group average body weights showed

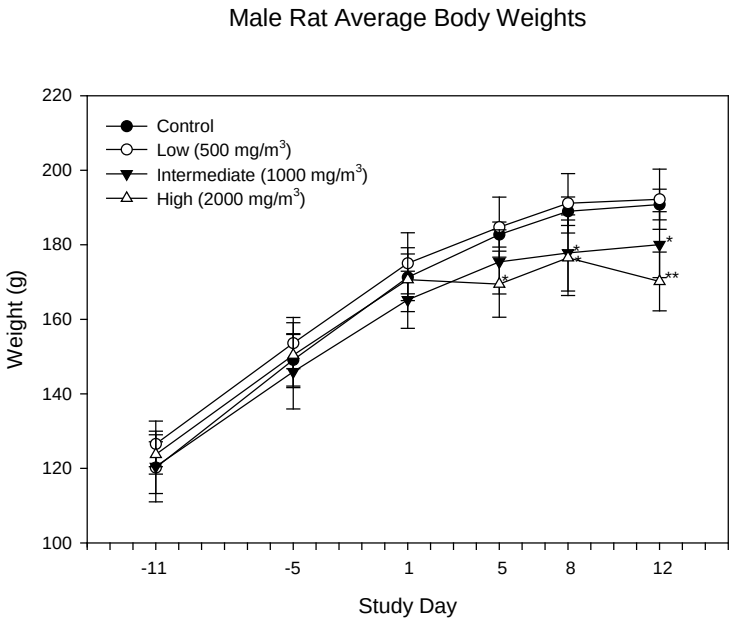
that the high concentration males at Study Days 5, 8 and 12, and the intermediate concentration males at Study Days 8 and 12, were significantly different from the control group. For the females, the average body weight for the high concentration group on some study days were lower than the controls; however, the differences from control weights were not statistically significant due to an unknown higher variability, as evidenced by greater standard deviations.

At the terminal necropsy, all of the exposure groups (including controls) had a lower average body weight than the average of the micronucleus groups, which were not exposed to FT jet fuel, and which were housed in polycarbonate cages (Figure 8).

The body weight trends discussed above are also illustrated by the body weight gain. The rate of weight gain decreased significantly during the weeks of exposure (Study Day 5 and 12) in both males and females (Figure 9) at the high (2000 mg/m^3) exposure concentration. The growth of male and female rats as reflected in body weight appeared to be related to the concentration of the jet fuel atmosphere. This most pronounced in the male and female body weight gain data (Figure 9), where the weight gain in the animals exposed to the highest concentration (2000 mg/m^3) is significantly lower immediately after each five day exposure period (Study Days 5 and 12). During the non-exposure weekend, those animals gained nearly as much as the animals exposed to lower concentrations and the controls (between Study Days 5 and 8).

There was one period of food consumption measurement which occurred during the weekend between exposures. The food consumption was consistent across the exposure groups, when compared with controls for females (Figure 10). For males, the intermediate concentration group consumed significantly less food when compared with controls, whereas the low and the high concentration groups were not statistically different. The weight gain for all animals was similar during the food consumption period. The evidence for a test compound-related effect on food consumption was weak.

a



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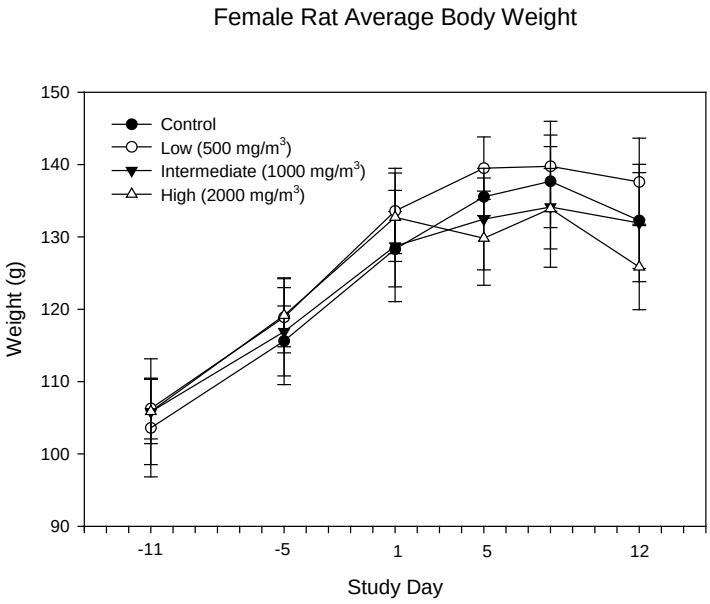


Figure 7. Ten-Day Exposure Body Weights as a Function of Study Day
Mean body weights (points) and their standard deviations (bars) are shown for (a) male and (b) female rats. Study Day 1 is the first day of exposure. Data were significantly different from control weights at the *$p < 0.05$ or **$p < 0.01$ levels, as noted.

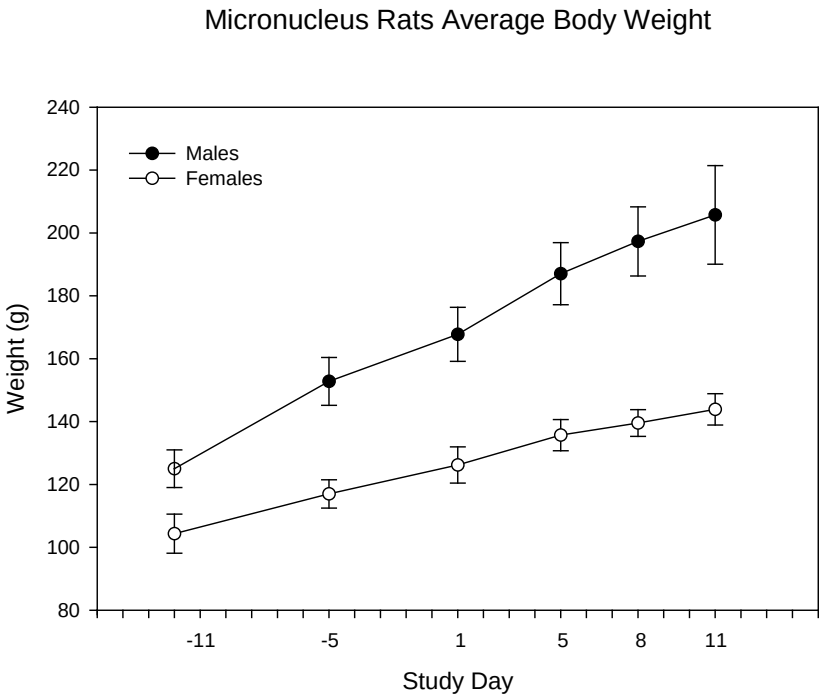
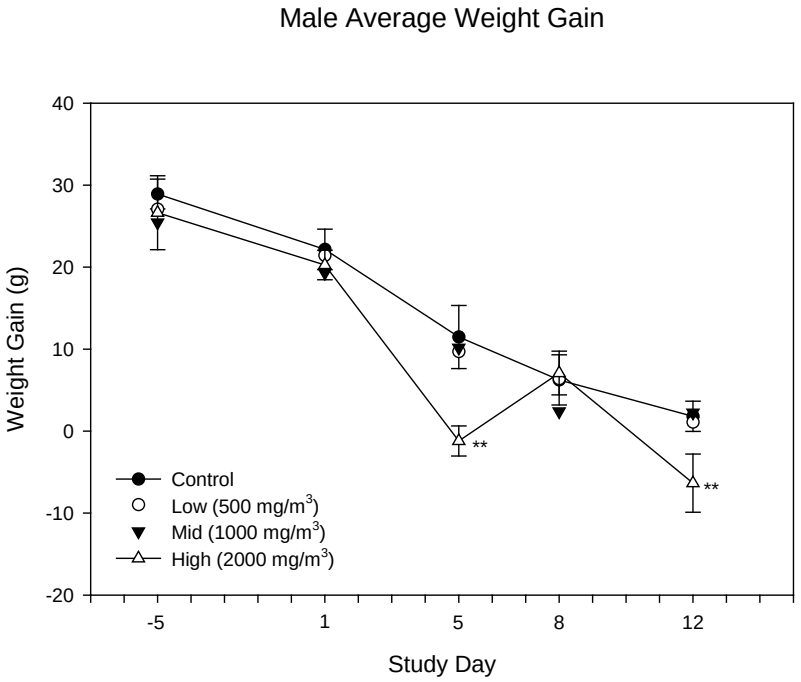


Figure 8. Micronucleus Control Group Rats Average Body Weights as a Function of Study Day

Study Day 1 is the first day of exposure. Points and bars represent means ± standard deviations.

a



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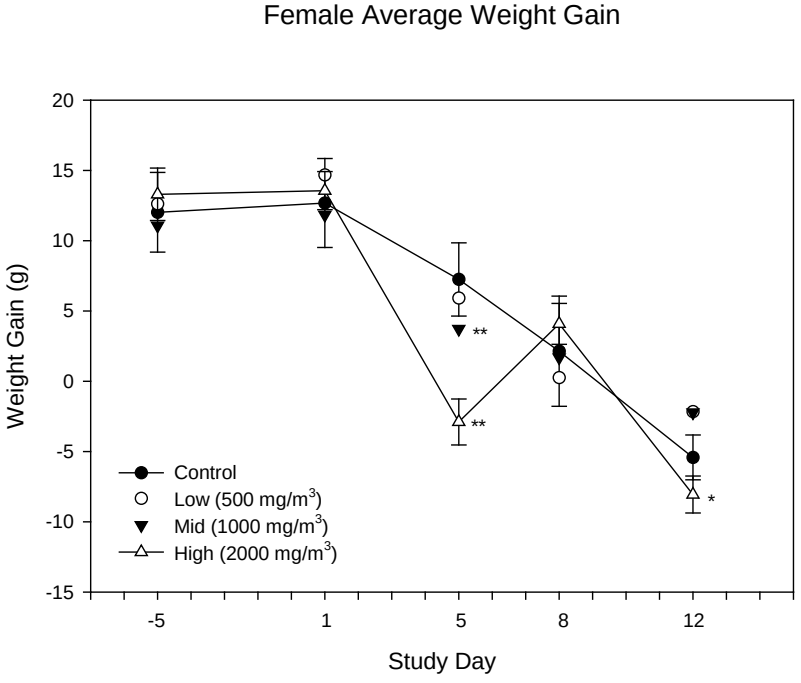

Figure 9. Ten-Day Exposure Average Weight Gain

Data shown are mean values (points) with standard deviations (bars) for (a) male and (b) female rats. Standard deviations are shown only for the control and high concentration groups. Data were significantly different from control gains at the *$p < 0.05$ or **$p < 0.01$ levels, as noted.

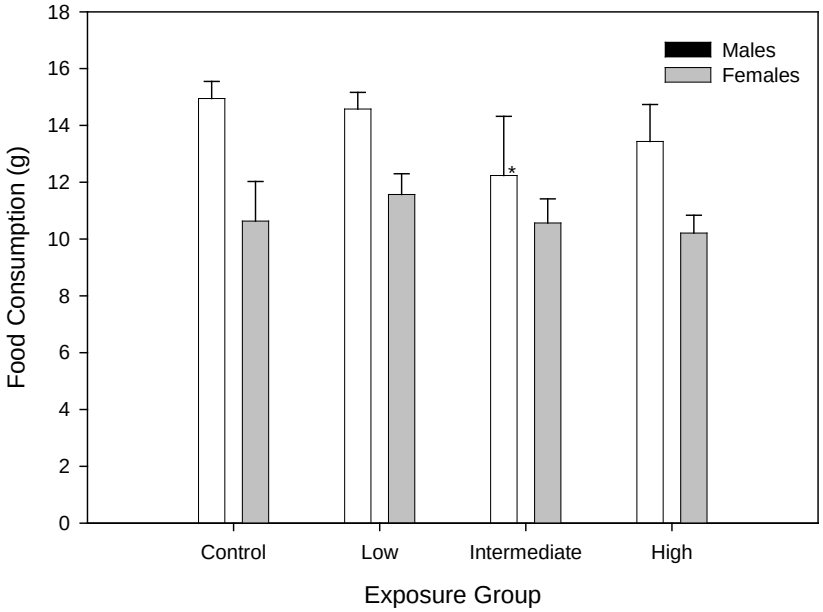

Figure 10. Ten-Day Exposure Group Weekend Food Consumption between Exposure Weeks

Data shown represent means ± standard deviations. The male food consumption for the intermediate (1000 mg/m^3) exposure group was determined to be statistically significant at the $p < 0.05$ level by a Dunnett's test (Provantis™, Conshohocken, PA).

4.4 Clinical Observations and Gross Pathology

4.4.1 Acute Exposure. Clinical observations were consistently normal over the course of the study. All animals in the acute exposure study survived until scheduled euthanasia. All animals were euthanized and necropsied on the same day. No gross pathology was observed. As no lesions or other unusual findings were observed, no further examinations were made and no tissues were taken, per guidelines.

4.4.2 Ten-Day Exposure. Over the course of the study, no unscheduled deaths occurred, nor were any animals found in a moribund condition. Clinical observations were conducted when animals were weighed. Observations included pelage alopecia in one female rat after a week of exposure at the low (500 mg/m^3) concentration, ear crust or exudate in one female rat after one week of exposure at the intermediate (1000 mg/m^3) concentration, and 3 of 5 female rats with a nasal discharge after a week of exposure at the high (2000 mg/m^3) concentration. In the male rats, the only clinical observation noted was nasal discharge in 5 of 5 rats after a week of exposure at the high (2000 mg/m^3) concentration.

In males exposed at the high (2000 mg/m^3) concentration, one animal had an incidental liver hernia, a lung focus and red discoloration around the nose. In two other males there was red discoloration around the nose. In females exposed at high concentration, one animal had a lung focus, one had red discoloration around the nose, and one had both lung foci and red discoloration around the nose. The observed lung foci were correlated to microscopic observations (see Section 4.6). One female exposed at low (500 mg/m^3) concentration had a kidney mass that was identified as a nephroblastoma. This was considered to be a spontaneous finding, not related to the exposure, based on the experience of the pathologist.

The adrenals, liver, kidney (right and left) and lungs were weighed in all jet fuel exposure animals at the terminal necropsy. The testes (right and left) from the male rats were also weighed. The average male liver weighed significantly less compared with control livers at the intermediate (1000 mg/m^3) and high concentrations (2000 mg/m^3). The liver was the only organ in males which showed a statistically significant effect attributable to jet fuel exposure (Table 9). Female liver weights did not show a statistically significant difference from controls. Females exposed to the high concentration had several organs that differed statistically from controls. The adrenal glands, right and left kidneys and lung all had an increased weight compared with controls.

Curiously, the left kidney, but not right kidney, was increased in weight for the intermediate and low (500 mg/m^3) concentration exposed females (Table 9). The significance of the increased kidney weights in females was unknown, since female rats do not accumulate hyaline droplets in kidney tissue (see Section 4.6). However, one female rat in the low concentration (500 mg/m^3) group had a much larger right kidney due to a spontaneous nephroblastoma that appeared to affect the statistics for this group (Table 9). In the intermediate (1000 mg/m^3) group, there was more variability in size for the right kidneys than the left. Mineralization, independent of treatment, was evident in both male and female rats in the cortex and in the medulla. The mineralization, which was more variable in the control and at low concentration compared to the intermediate and high concentrations, may also be responsible for the differences in kidney weights in the females.

Table 9. Ten-Day Exposure Group Average Organ Weights

Males

Organ	Control	Low (500 mg/m^3)	Intermediate (1000 mg/m^3)	High (2000 mg/m^3)
Terminal Body Weight	190.80 ± 4.12	192.23 ± 8.08	180.04 ± 8.86	170.17 ± 7.90
Adrenal Gland, bilateral	0.0376 ± 0.0048	0.0388 ± 0.0044	0.0372 ± 0.0029	0.0376 ± 0.0050
Kidney, Left	0.680 ± 0.034	0.714 ± 0.029	0.696 ± 0.069	0.680 ± 0.032
Kidney, Right	0.6870 ± 0.0305	0.7022 ± 0.0520	0.6556 ± 0.0437	0.6746 ± 0.0391
Liver	7.268 ± 0.443	7.084 ± 0.475	*6.606 ± 0.539	**6.366 ± 0.181
Lung	0.9200 ± 0.0741	0.9008 ± 0.0569	0.8674 ± 0.0620	0.9214 ± 0.1016
Testis, Left	1.2902 ± 0.0422	1.2858 ± 0.0340	1.1988 ± 0.1030	1.2652 ± 0.0633
Testis, Right	1.2672 ± 0.0516	1.2382 ± 0.0460	1.1516 ± 0.1030	1.2168 ± 0.0831

Females

Organ	Control	Low (500 mg/m^3)	Intermediate (1000 mg/m^3)	High (2000 mg/m^3)
Terminal Body Weight	132.26 ± 6.63	137.60 ± 6.04	131.92 ± 8.11	125.84 ± 5.91
Adrenal Gland, bilateral	0.0434 ± 0.0026	0.0502 ± 0.0065	0.0442 ± 0.0041	*0.0510 ± 0.0045
Kidney, Left	0.490 ± 0.034	*0.540 ± 0.029	*0.522 ± 0.008	*0.526 ± 0.025
Kidney, Right	0.4878 ± 0.0306	0.6116 ± 0.2046	0.5152 ± 0.0263	*0.5318 ± 0.0244
Liver	4.314 ± 0.278	4.808 ± 0.496	4.468 ± 0.237	4.570 ± 0.220
Lung	0.7266 ± 0.0734	0.8144 ± 0.0682	0.7616 ± 0.0595	*0.8596 ± 0.1009

Notes: Weight in grams, mean ± standard deviation; *Different from Control group at the $p < 0.05$ significance level; **Different from Control group at the $p < 0.01$ significance level

4.5 Ten-Day Exposure - Histopathology

No adverse effects were observed in histological sections of the trachea, larynx, spleen, adrenals, or heart. In the liver, hepatocyte hypertrophy was observed in all exposure concentrations of males, and in the highest exposure concentration in females. The hypertrophy was characterized by an increase in the cytoplasmic compartment of the hepatocytes with a corresponding compression of liver sinusoids. The cytoplasm had a ground glass appearance. These observations were consistent with cytochrome P450 induction, and were considered to be an adaptive change and not a toxic response to the inhaled test material.

In the kidneys, males in all exposure groups showed an accumulation of hyaline droplets in the proximal convoluted tubular cells. Hyaline droplets were not observed in the female kidney. Hyaline droplets are consistent with the accumulation of a male rat-specific protein, alpha-2µ-globulin in the kidney. Production of alpha-2µ-globulin begins in the male rat after puberty. Accumulation of alpha-2µ-globulin in the male rat kidney may be enhanced by exposure to an inducer (Alden, 1986; Flamm and Lehman-McKeeman, 1991). Hyaline droplets were scored as mild to slight in all of the jet-fuel exposed male groups, and did not show a correlation with jet fuel exposure concentration. In the proximal convoluted tubules of control males, eosinophilic droplets were observed and were considered to be a normal background finding in male kidneys. In both male and female rat kidneys, discrete foci of mineralization were seen in the cortex and medulla, independent of treatment.

Minimal to mild olfactory epithelial degeneration was observed in the nasal tissues of male and female rats at the intermediate (1000 mg/m^3) and high (2000 mg/m^3) concentrations. The effects increased with increasing concentration, and with deeper penetration into the nose. For example, Level III and IV showed mild effects while Level II showed minimal effects (Figure 11). Nasopharyngeal duct goblet cell hypertrophy or hyperplasia was observed in Level IV at the highest concentration in both male and female rats.

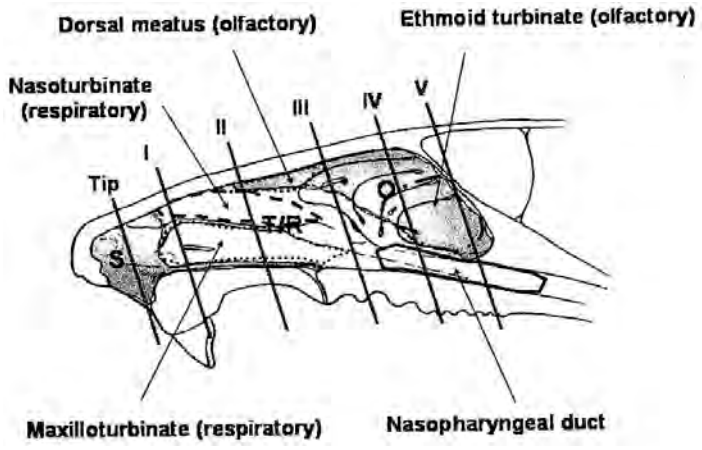

Figure 11. Nasal Passages of the Rat and Section Levels for Histopathology
Note: Adapted from Morgan (1991; Figures 3 and 4)

In the lungs, at the high (2000 mg/m^3) concentration in both male and female rats, there were multifocal areas of inflammatory cell infiltration, typically neutrophils and alveolar histocytes throughout the alveoli. Animals exposed at the intermediate (1000 mg/m^3) concentration showed a lesser effect. The inflammatory foci appeared to be correlated to the lung foci seen in the gross observations.

Details of the histopathology results and specific incidences of the observed effects are provided in the histopathology report, Appendix B.

4.6 Genotoxicity: Micronucleus Induction

The results of the CP administration as a positive control and saline as a negative vehicle control showed a 5 to 9-fold increase in micronuclei frequency over saline controls in males and females, indicating that this batch of F344 rats was responsive to a known micronuclei inducer. For rats exposed to jet fuel, the ratio of PCEs to NCEs did not indicate any toxicity of the jet fuel to the bone marrow cells. The frequency of micronuclei in the jet fuel-exposed animals showed that there was no evidence for induction of micronuclei in males or females at any of the exposure concentrations. Therefore, based on the lack of induction of micronuclei, these results indicate that FT jet fuel was not genotoxic.

Details of the micronucleus study results are provided in the report attached in Appendix C.

4.7 Stability and Fingerprint Analyses

The FT jet fuel with additives was used neat. The results of the gas chromatographic analysis conducted at WPAFB of the jet fuel samples show similar chromatograms between the sample of the drum taken after the completion of the acute inhalation exposure, the ten-day inhalation study and the sample from the original stock material at WPAFB. At the level of resolution between the chromatograms, no change between jet fuel samples was observed, which indicates that the FT jet fuel with additives was stable (Figure 12).

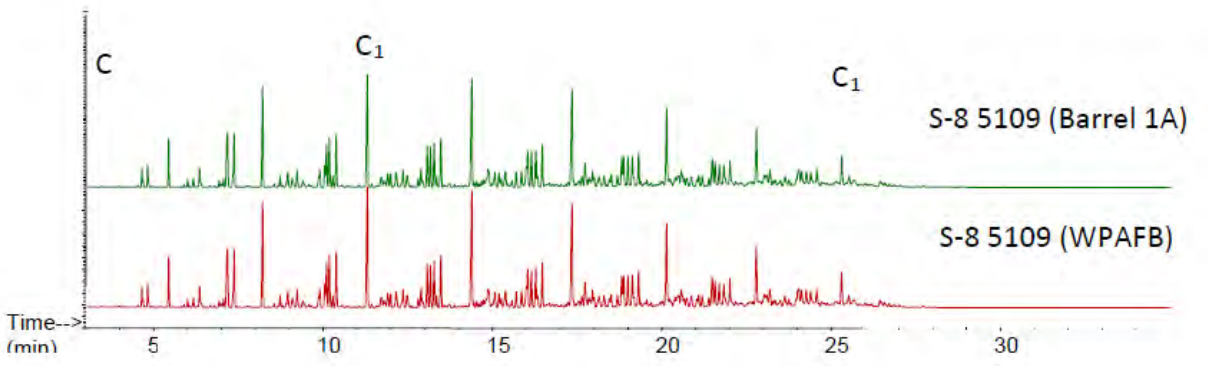

Figure 12. Stability of FT Jet Fuel with Additives
Chromatograms of samples taken from the jet fuel drum (Barrel 1A) and from a sample of the stock material from WPAFB show similar patterns.

The results of the assay by gas chromatography and mass spectrometry to identify major components of the jet fuel confirmed that the FT jet fuel with additives was a complex mixture of C8 to C16 aliphatic compounds. Complete results are found in Appendix D. The following conclusions can be drawn from this analysis.

1. Aerosol/vapor fraction analysis demonstrated an increased presence of high molecular weight compounds in the aerosol phase compared to an increased presence of low molecular weight compounds in the vapor phase.

2. When comparing the aerosol phases of the different concentration groups, there was no appreciable difference in the distribution of compounds among them.

3. In the vapor phase, there appeared to be more total compounds present in the high concentration exposure samples compared to the low concentration exposure samples.

4. A majority of the compounds (accounting for >90 percent of the total peak area in the sample) found in the low concentration vapor samples were those found between n-undecane and n-tetradecane. The high concentration vapor samples, meanwhile, appeared to contain a much larger range of molecular weight compounds (i.e., n-octane through n-pentadecane).

5.0 DISCUSSION AND CONCLUSIONS

An acute study was conducted with five male and five female Fischer 344 rats exposed to a synthetic jet fuel (FT jet fuel with additives) by inhalation. The FT jet fuel with additives was generated as a mixture of aerosol and vapor at an average total concentration of 2044 mg/m^3. The aerosol concentration averaged 596 mg/m^3. The exposure was four hours long. Animals were held for 14 days post-exposure. There was an indication of a reduced body weight gain in the days following the exposure. Clinical observations of the animals were normal throughout the study. There were no gross pathological observations at the final necropsy. As no lethality was observed in this study, no further acute inhalation testing was indicated. The exposure concentration of FT jet fuel with additives used in this study was then considered and chosen for use in longer duration dose response studies. The results of the acute inhalation limit test are consistent with the results for other jet fuels. No signs of toxicity were observed in male and female rats exposed to JP-8 vapor only (3430 mg/m^3) or vapor plus aerosol (4440 mg/m^3) in the 4-hour test (Wolfe *et al.*, 1996). The acute inhalation limit test value (no observed acute toxicity) for petroleum JP-4 was 5140 mg/m^3 and 5,010 mg/m^3 for shale JP-4 (Clark *et al.* 1989).

A ten-day short term study was conducted with male and female Fischer 344 rats exposed to a synthetic jet fuel by inhalation. This study was performed to help select the concentrations for a planned 90-day exposure. The jet fuel was generated as a mixture of aerosol and vapor at three target concentrations (500, 1000 and 2000 mg/m^3) and a control with clean air. Each exposure group consisted of five male and five female rats. Exposures were conducted for six hours/day, five days per week for two weeks. A slight effect on bodyweight was observed, with males and females exposed to the high (2000 mg/m^3) concentration weighing 11 and 5 percent less than controls, respectively. The decreases were statistically significant for males but not for females. Food consumption differences did not appear to be dose-related. Animals were necropsied immediately after exposure and examined for gross lesions. Target tissues were examined histopathologically. No adverse effects were seen in the trachea, larynx, spleen, adrenals and heart. In the lung, foci of inflammatory cell infiltration were observed in the high concentration group and somewhat in the intermediate group. Hepatocyte hypertrophy, consistent with

cytochrome P450 induction, was observed in all male groups and the high concentration female group. This observation was considered to be adaptive. Hyaline droplet accumulation was observed in the kidney cells of all male rats exposed to FT jet fuel.

Ten-day studies for JP-8 were performed previously; the reports are in the writing process. Therefore, it is not possible to compare FT jet fuel and JP-8 short-term toxicity at this time.

The micronucleus assay indicated that the F344 rats used in the study responded appropriately to positive and negative controls. Toxicity of FT jet fuel to bone marrow cells was not indicated by this study. FT was evaluated previously in two other genotoxicity assays. The Ames *Salmonella* gene mutation assay was negative, as was the chromosome aberration assay with human lymphocytes *in vitro* (Mattie *et al.*, 2011). Taken alone or together, these three assays indicate that FT jet fuel is not likely to be genotoxic.

JP-8 was also evaluated in three genotoxicity assays. However, there was only one common assay, the Ames *Salmonella* gene mutation assay, used for both JP-8 and FT jet fuel. JP-8 was also tested in the mouse lymphoma assay for gene mutation and in the unscheduled DNA synthesis assay in mouse cells. For all three assays, no evidence was found to indicate that JP-8 is genotoxic (Brusick and Matheson, 1978).

The fingerprint analysis of the aerosol and vapor phase of the delivered test chemical mixture determined the hydrocarbon fingerprint of the FT jet fuel in the chambers by GC/MS. No appreciable difference was found in the distribution of compounds in the aerosol from each concentration group. However, in the vapor phase, more total compounds were present in the high concentration exposure samples compared to the low concentration exposure samples. The majority of the compounds (accounting for >90 percent of the total peak area in the sample) found in the low concentration vapor samples were those found between n-undecane and n-tetradecane. In contrast, the high concentration vapor samples appeared to contain a much larger range of molecular weight compounds (i.e., n-octane through n-pentadecane was observed).

Overall, these studies indicate that FT jet fuel is similar to or less toxic than JP-8. Additional toxicity studies with FT jet fuel, including a 90-day inhalation study and a sensory irritation study, have been performed and are being reported elsewhere.

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APPENDIX A. TABULAR RESULTS

Table 1. Acute Exposure Body Weights

Male Rats by Animal Number								
Day	**1**	**2**	**3**	**4**	**5**	**Group Average**	**SD**	**CV (%)**
-21	124.0	117.4	111.8	122.1	105.1	116.1	7.7	6.7
-13	173.0	166.0	158.8	175.1	154.2	165.4	9.0	5.4
-10	186.0	177.9	174.0	189.8	169.2	179.4	8.5	4.7
-7	194.7	186.8	187.0	201.6	178.0	189.6	8.9	4.7
0	211.6	206.3	209.8	222.3	196.6	209.3	9.3	4.4
7	213.2	214.4	219.3	230.9	211.6	217.9	7.8	3.6
13	237.5	238.7	242.8	257.1	238.0	242.8	8.3	3.4
Female Rats by Animal Number								
Day	**6**	**7**	**8**	**9**	**10**	**Group Average**	**SD**	**CV (%)**
-21	115.4	112.3	116.3	114.5	115.6	114.8	1.5	1.3
-13	134.1	133.3	134.1	130.9	136.0	133.7	1.8	1.4
-10	138.2	137.7	140.2	136.8	141.3	138.8	1.9	1.3
-7	140.8	140.8	142.7	141.2	143.4	141.8	1.2	0.8
0	148.5	149.2	149.7	150.8	156.1	150.9	3.0	2.0
7	150.0	149.0	151.6	153.9	154.3	151.8	2.3	1.5
13	164.6	164.9	163.8	165.4	169.5	165.6	2.2	1.3

Notes: weights in grams; SD = standard deviation; CV = coefficient of variation

Table 2. Acute Exposure Average Body Weight Gain

Male Rats by Animal Number								
Day	**1**	**2**	**3**	**4**	**5**	**Group Average**	**SD**	**CV (%)**
-13	6.1	6.1	5.9	6.6	6.1	6.2	0.28	4.5
-10	4.3	4.0	5.1	4.9	5.0	4.7	0.48	10.3
-7	2.9	3.0	4.3	3.9	2.9	3.4	0.67	19.7
0	2.4	2.8	3.3	3.0	2.7	2.8	0.32	11.3
7	0.2	1.2	1.4	1.2	2.1	1.2	0.68	56.7
13	4.1	4.1	3.9	4.4	4.4	4.2	0.21	5.2
Female Rats by Animal Number								
Day	**6**	**7**	**8**	**9**	**10**	**Group Average**	**SD**	**CV (%)**
-13	2.3	2.6	2.2	2.1	2.6	2.4	0.24	10.0
-10	1.4	1.5	2.0	2.0	1.8	1.7	0.30	17.2
-7	0.9	1.0	0.8	1.5	0.7	1.0	0.30	30.3
0	1.1	1.2	1.0	1.4	1.8	1.3	0.32	24.7
7	0.2	0.0	0.3	0.4	-0.3	0.1	0.27	213.0
13	2.4	2.7	2.0	1.9	2.5	2.3	0.32	13.9

Note: gain in grams/day

Table 3. Ten-Day Exposure Group Average Body Weights

Males

Study Day	Control	Low (500 mg/m^3)	Intermediate (1000 mg/m^3)
-11	120.20 ± 6.95	126.56 ± 6.12	120.50 ± 9.49
-5	149.12 ± 7.04	153.62 ± 6.89	145.94 ± 10.01
1	171.28 ± 6.25	175.04 ± 8.19	165.24 ± 7.67
5	182.76 ± 3.36	184.74 ± 8.07	175.40 ± 8.60
8	189.00 ± 3.83	191.14 ± 7.97	*177.80 ± 10.22
12	190.80 ± 4.12	192.23 ± 8.07	*180.04 ± 8.86

Study Day	High (2000 mg/m^3)	MN
-11	123.74 ± 5.27	125.00 ± 6.00
-5	150.38 ± 8.72	152.79 ± 7.61
1	170.64 ± 8.57	167.73 ± 8.59
5	*169.44 ± 8.86	187.00 ± 9.87
8	*176.52 ± 10.13	197.31 ± 11.00
12	**170.17 ± 7.90	[a]205.74 ± 15.67

Females

Study Day	Control	Low (500 mg/m^3)	Intermediate (1000 mg/m^3)
-11	103.60 ± 6.77	106.28 ± 4.20	105.84 ± 7.32
-5	115.62 ± 4.83	118.90 ± 4.09	116.90 ± 7.32
1	128.30 ± 5.20	133.58 ± 5.90	128.74 ± 7.69
5	135.54 ± 2.61	139.50 ± 4.32	132.46 ± 7.02
8	137.68 ± 6.39	139.76 ± 6.22	134.14 ± 8.34
12	132.26 ± 6.63	137.60 ± 6.04	131.92 ± 8.11

Study Day	High (2000 mg/m^3)	MN
-11	105.86 ± 4.44	104.35 ± 6.23
-5	119.16 ± 5.18	116.99 ± 4.51
1	132.72 ± 6.10	125.19 ± 4.18
5	129.82 ± 6.50	135.69 ± 4.96
8	133.90 ± 5.57	139.56 ± 4.26
12	125.84 ± 5.90	[a]143.88 ± 4.97

Notes: Weights are in grams, mean ± standard deviation; [a]Terminal weights of MN animals were taken on Study Day 11; *Different from Control group at the $p < 0.05$ significance level; **Different from Control group at $p < 0.01$ significance level

Table 4. Ten-Day Exposure Average Body Weight Gain

Males Study Day	Control	Low (500 mg/m^3)	Intermediate (1000 mg/m^3)	High (2000 mg/m^3)
-5	28.92 ± 1.82	27.06 ± 4.37	25.44 ± 3.06	26.64 ± 4.51
1	22.16 ± 2.47	21.42 ± 1.89	19.30 ± 2.62	20.26 ± 1.81
5	11.48 ± 3.85	9.70 ± 3.19	10.16 ± 2.47	*-1.20 ± 1.83
8	6.24 ± 3.06	6.40 ± 3.13	2.40 ± 2.18	7.08 ± 2.67
12	1.80 ± 1.84	1.09 ± 0.97	2.24 ± 1.63	*-6.35 ± 3.55
Females Study Day	Control	Low (500 mg/m^3)	Intermediate (1000 mg/m^3)	High (2000 mg/m^3)
-5	12.02 ± 2.84	12.62 ± 1.59	11.06 ± 3.19	13.30 ± 1.86
1	12.68 ± 3.16	14.68 ± 2.16	11.84 ± 0.98	13.56 ± 1.36
5	7.24 ± 2.61	5.92 ± 1.99	**3.72 ± 1.64	**-2.90 ± 1.63
8	2.14 ± 3.92	0.26 ± 2.35	1.68 ± 1.90	4.08 ± 1.46
12	-5.42 ± 1.59	-2.16 ± 2.01	-2.22 ± 1.56	*-8.06 ± 1.31

Notes: Weight gain in grams, mean ± standard deviation; *Different from Control group at the $p < 0.05$ significance level; **Different from Control group at the $p < 0.01$ significance level

Table 5. Ten-Day Exposure Group Average Food Consumption

	Control	Low (500 mg/m^3)	Intermediate (1000 mg/m^3)	High (2000 mg/m^3)
Male	14.95 ± 0.60	14.58 ± 0.58	*12.24 ± 2.08	13.44 ± 1.30
Female	10.63 ± 1.39	11.57 ± 0.73	10.57 ± 0.85	10.21 ± 0.62

Notes: Weight gain in grams, mean ± standard deviation; *Different from Control group at the $p < 0.05$ significance level

APPENDIX B. TWO-WEEK INHALATION TOXICITY STUDY OF FT JET FUEL IN F344 RATS: PATHOLOGY REPORT

THE HAMNER INSTITUTES FOR HEALTH SCIENCES STUDY: 08002

EPL PROJECT NO.: 304-432

PATHOLOGY REPORT

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October 12, 2009

INTRODUCTION

S-8 (or FT) jet fuel is a synthetic organic mixture produced using the Fischer-Tropsch (FT) process that converts carbon monoxide and hydrogen to liquid hydrocarbons. FT jet fuel is being developed to replace or augment petroleum-derived JP-8 jet fuel for military use by the U.S. armed forces. JP-8 fuel contains a mixture of aliphatic and aromatic hydrocarbons. The FT process for FT creates a mixture of aliphatic compounds similar to those found in JP-8 but does not form aromatic benzene and naphthalene compounds. This difference of composition between FT and JP-8 fuel points to a potential difference in the toxicity of the two fuels.

During refueling operations, personnel may be exposed to vapors and aerosols of jet fuel primarily by dermal or inhalation exposure. A recent review of JP-8 jet fuel toxicology concluded that exposure to JP-8 near the permissible exposure limit (PEL) of 350 mg/m^3 was potentially toxic to the immune, respiratory and nervous systems (NRC, 2003). In the respiratory system, physiological effects on pulmonary function and cellular effects have been observed (Herrin *et al.*, 2006) from exposure to JP-8.

Very limited toxicity testing of FT has been performed. Since inhalation is a major route of exposure for JP-8 jet fuel, the assessment of toxicity of FT by inhalation is needed to assess the risk of replacing or augmenting JP-8 by FT fuel. This study was designed to assess the potential inhalation toxicity of a test substance when administered via inhalation exposure to Fischer 344 rats on a repeated basis for five days per week over two weeks. The assessment included clinical observations, gross pathology and histopathology. Additionally, there was a set of animals for an erythrocyte micronuclei assay of DNA damage.

This Hamner Institutes for Health Sciences Study 08002- was conducted in male and female F344 rats. The experimental design and animal identification are summarized in Tables 1 and 2 below:

Table 1. Experimental Design

Group	Exposure Level (mg/m^3)	Number of Animals	
		Males	Females
Control	0	5	5
Low	500	5	5
Intermediate	1000	5	5
High	2000	5	5
Micronuclei Neg.control	NA	5	5
Micronuclei Pos. control	NA	5	5
Total		30	30

Table 2. Animal Identification

Group	Exposure Level (mg/m^3)	Animal Identification Number 08002-	
		Males	Females
Control	0	101-105	201-205
Low	500	106-110	206-210
Intermediate	1000	111-115	211-215
High	2000	116-120	216-220
Micronuclei Neg. control	NA	121-125	221-225
Micronuclei Pos. control	NA	126-130	226-230
Total		30	30

Inhalation Exposures

The animals were exposed by inhalation via whole-body exposure. The test substance was administered for approximately six hours per exposure day, five days per week over a two week period.

The in life phase of the study and the necropsies were performed at the Hamner Institute. Dr. G. Willson of EPL, NC, supervised the necropsy which took place on March 7, 2008.

Necropsy Procedures

Animals to be euthanized were deeply anesthetized with sodium pentobarbital (intraperitoneal injection, approximately 30 mg/kg) and exsanguinated by transection of the abdominal aorta.

A complete macroscopic examination was performed on all animals. The necropsy included examination of the external surface and all orifices; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass.

HISTOPATHOLOGY PROCEDURES

Histological sections of the tissues listed below were stained with hematoxylin and eosin (H&E) and evaluated via light microscopy.

- Trachea
- Larynx
- Lungs (two sections)
- Liver (two sections)
- Kidneys
- Spleen
- Adrenals
- Heart
- Nasal cavity (four sections)

Histological evaluation of the high concentration and control groups of animals was done initially. When a treatment-related effect was observed in the high-dose group, tissues from the intermediate groups were examined to establish a no observable effect level (NOEL).

During the light microscopic examination histopathologic diagnoses for tissues of each animal were recorded. Microscopic findings were graded using a subjective grading scale (1 =minimal, 2=slight/mild, 3=moderate, 4=moderately severe, 5=severe/high). After individual animal histopathology findings were reviewed, incidence tables that summarized histopathology findings by treatment groups were prepared.

RESULTS AND DISCUSSION

All study animals survived until the scheduled necropsy. There were several gross findings. In the high-dose males, there was an incidental liver hernia in one animal, lung foci in one animal and red discoloration around the nose in three animals. In the high-dose females, there were two animals with lung foci and two animals with red discoloration around the nose. The lung foci were considered to correlate with the inflammatory foci evident histologically. The kidney mass evident in a low dose female was a nephroblastoma histologically.

Tissues were examined from high dose and control animals and when a potential treatment-related effect was determined, these tissues were also examined from the low and intermediate exposure animals. Incidence tables are included in Appendix I.

Trachea

No adverse effects were observed.

Larynx

No adverse effects were observed.

Lungs

At the highest exposure level there were multifocal areas of inflammatory cell infiltration throughout the alveoli. In these areas there were typically neutrophils and alveolar histiocytes. The larger areas sometimes exhibited a granulomatous appearance with central histiocyte aggregation and peripheral neutrophils. A lesser effect was seen at the intermediate exposure level (Tables 3 and 4).

Table 3. Incidence of Inflammatory Cell Infiltration in the Lungs of Male Rats (5 rats per group)

Group	Multifocal Inflammatory Cell Infiltration	Focal Inflammatory Cell Infiltration
control	0	0
low	0	0
intermediate	1	1
high	5	0

Table 4. Incidence of in Inflammatory Cell Infiltration in the Lungs of Female Rats (5 rats per group)

Group	Multifocal Inflammatory Cell Infiltration	Focal Inflammatory Cell Infiltration
control	0	0
low	0	0
intermediate	0	2
High	5	0

Liver

Hepatocyte hypertrophy was evident in all exposure groups of males but only the highest exposure group of females. Although this was pan lobular it was more evident in the periportal region. There was an increase in the cytoplasmic compartment of the hepatocytes and compression of sinusoids. Hepatocyte cytoplasm had a ground glass appearance. These findings are typically associated with cytochrome P450 induction and are considered an adaptive change (Eustis, 1990).

Kidneys

All males exposed to jet fuel exhibited an increased accumulation of hyaline droplets in the proximal convoluted tubular cells. The droplets were of varying increased sizes, some larger droplets had an angular appearance, and occasionally tubular cells were enlarged. Occasional degenerative epithelial cells within these tubules had crisply eosinophilic cytoplasm and pyknotic nuclei. All treated animals had a mild accumulation of hyaline droplets. Control males had some eosinophilic droplets within proximal convoluted tubules. These were characterized as discrete smaller droplets within the cytoplasm and were basally located. These eosinophilic droplets are normally encountered in male kidneys at this age and are considered a background finding.

Discrete foci of mineralization were evident in both male and female rats in the cortex and medulla and were independent of treatment. A nephroblastoma was present in a low-dose female. This is considered to be a spontaneous finding.

Spleen

No adverse effects were observed.

Adrenals

No adverse effects were observed.

Heart

No adverse effects were observed.

Nasal Cavity

Olfactory epithelial degenerative changes were evident in the noses of rats exposed to the high and intermediate dose of jet fuel in both males and females. There was also an effect on the transitional epithelium in the anterior nose and the respiratory epithelium in the nasopharyngeal duct. The nasal effects were more severe in the highest exposure group than in the intermediate dose exposure group (Tables 5 and 6). Below are brief descriptions of the lesions as they occurred in the different nose sections:

Level I

Minimal hyperplasia of the epithelium of the lateral wall was evident in both male and female rats at the highest exposure level.

Level II

Minimal olfactory epithelial degeneration on the septum and lateral wall. This occurred usually at the junction of the respiratory and olfactory epithelium.

Level III

Mild olfactory epithelial degeneration. Multifocal affecting areas of the septum and parts of the turbinates.

Level IV

Mild olfactory epithelial degeneration which is multifocal affecting areas of the septum and parts of the turbinates. Nasopharyngeal duct goblet cell hypertrophy/hyperplasia is evident in both sexes of rats exposed to the two highest doses of jet fuel.

Table 5. Incidence of Olfactory Epithelial Degeneration in Male Rats (5 rats per group)

Group	Level II	Level III	Level IV
control	0	0	0
low	0	0	0
intermediate	2	2	0
high	4	5	5

Table 6. Incidence of Olfactory Epithelial Degeneration in Female Rats (5 rats per group)

Group	Level II	Level III	Level IV
control	0	0	0
low	0	0	0
intermediate	2	4	4
high	5	5	4

CONCLUSIONS

Exposure of rats by inhalation to jet fuel induced histological findings in lung, nose and liver and, in male rats, kidneys.

Inflammatory foci were evident in the lungs of both sexes of rat exposed to the two highest doses of jet fuel. Olfactory epithelial degeneration was evident in both sexes of rats exposed to the two highest doses of jet fuel.

An increase in hyaline droplet accumulation was seen in kidneys of all male rats exposed to jet fuel. This finding is characteristic of an alpha-2μ-globulin inducer (Montgomery, 1990).

Liver changes were evident at all exposure levels in the males and the high dose only in the females. This panlobular hepatocyte change reflects an adaptive response to the test material.

GABRIELLE A. WILLSON, B.V.M.S., MRCVS
F.R.C. Path
Pathologist

12th October 2009
Date

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QUALITY ASSURANCE FINAL CERTIFICATION

Study Title: Two-Week Inhalation Toxicity Study of FT Jet Fuel in Rats

Client Study: Protocol No. 08002 EPL Principal Investigator: Dr. Gabrielle Willson

EPL Project Number: 304-432 EPL Pathologist: Dr. Gabrielle Willson

The following aspects of this study were inspected by the Quality Assurance Unit of Experimental Pathology Laboratories, Inc. Dates inspections were performed and findings reported to the EPL Principal Investigator and Management are indicated below.

	Dates	
Area Inspected	Inspection	Reporting
EPL Project Sheets	February 19, 2008; February 29, 2008; September 10, 2008	February 19, 2008; February 29, 2008; September 10, 2008
Project Setup	March 10, 2008; March 11, 2008	March 10, 2008; March 11, 2008
Data Review	March 18, 2008; March 19, 2008	March 18, 2008; March 19, 2008
Draft Pathology Report	April 9, 2008	April 9, 2008
Final Pathology Report	October 9, 2009	October 9, 2009

Date reported to Study Director/Management: October 9, 2009

Date of last quarterly facility inspection: July 2009

David J Hollenbach
EPL Quality Assurance Unit

October 13, 2009
Date

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COMPLIANCE STATEMENT

Client Name	The Hamner Institutes for Health Sciences	EPL Project Coordinator/ Principal Investigator	Dr. Gabrielle Willson
Client Study	Protocol No. 08002	EPL Pathologist	Dr. Gabrielle Willson
Species	F344 Rats	EPL Project Number	304-432
Study Title	Two-Week Inhalation Toxicity Study of FT Jet Fuel in Rats		
Test Article	FT Jet Fuel		

The histopathology procedures for this study were completed in accordance with applicable standard operating procedures, the protocol, all amendments, and the protocol-specified EPA Toxic Substances Control Act (TSCA) Good Laboratory Practices (GLP) regulations as noted in 40 CFR 792.

GABRIELLE A. WILLSON, B.V.M.S., MRCVS
F.R.C. Path

12th October 2009
Date

APPENDIX I: INCIDENCE TABLES

SUMMARY INCIDENCE TABLE

08002
Two-Week Sacrifice
Male Rat

	GROUP 1	GROUP 2	GROUP 3	GROUP 4		
ADRENAL (NO. EXAMINED)	(5)			(5)		
HEART (NO. EXAMINED)	(5)	(1)		(5)		
Cardiomyopathy	2			2		
KIDNEY (NO. EXAMINED)	(5)	(5)	(5)	(5)		
Nephroblastoma						
Hyaline Droplet, Tubular						
Epithelium		5	5	5		
Infiltrate, Mononuclear Cells			2			
Mineralization, Cortical	5	4	5	5		
Mineralization, Medullary	3	5	4	4		
Regeneration, Tubular						
Epithelium	1	2		1		
LARYNX (NO. EXAMINED)	(5)			(5)		
LIVER (NO. EXAMINED)	(5)	(5)	(5)	(5)		
Hypertrophy		5	5	5		
Inflammation, Chronic, Focal	2		2	1		
LUNG (NO. EXAMINED)	(5)	(5)	(5)	(5)		
Alveolar Epithelium,						
Hyperplasia				3		
Alveolar Histiocytosis		1	1			
Alveoli, Inflammatory Cell						
Infiltration, Focal			1			
Alveoli, Inflammatory Cell						
Infiltration, Multifocal			1	5		
Interstitium, Fibrosis	1					
Subpleural Lymphocytes	2	2	4	2		
NASAL TURB LEVEL I (NO.						
EXAMINED)	(5)	(5)	(5)	(4)		
Respiratory Epithelium,						
Hyperplasia			1	4		
Respiratory Epithelium,						
Inflammation		1	2			
NASAL TURB LEVEL II (NO.						
EXAMINED)	(5)	(5)	(5)	(5)		
Olfactory Epithelial						
Degeneration			2	4		

SUMMARY INCIDENCE TABLE

08002
Two-Week Sacrifice
Male Rat

	GROUP 1	GROUP 2	GROUP 3	GROUP 4		
NASAL TURB LEVEL III (NO.						
EXAMINED)	(5)	(5)	(5)	(5)		
Olfactory Epithelial						
Degeneration			2	5		
NASAL TURB LEVEL IV (NO.						
EXAMINED)	(5)	(5)	(5)	(5)		
Nasopharyngeal Duct, Goblet						
Cell Hyperplasia/Hypertrophy			5	5		
Olfactory Epithelial						
Degeneration				5		
SPLEEN (NO. EXAMINED)	(5)			(5)		
TRACHEA (NO. EXAMINED)	(5)			(5)		

SUMMARY INCIDENCE TABLE

08002
Two-Week Sacrifice
Female Rat

	GROUP 1	GROUP 2	GROUP 3	GROUP 4		
ADRENAL (NO. EXAMINED)	(5)			(5)		
HEART (NO. EXAMINED)	(5)			(5)		
Cardiomyopathy				2		
KIDNEY (NO. EXAMINED)	(5)	(5)	(5)	(5)		
Nephroblastoma		1				
Hyaline Droplet, Tubular						
Epithelium						
Infiltrate, Mononuclear Cells						
Mineralization, Cortical	5	2	5	5		
Mineralization, Medullary	3	5	5	5		
Regeneration, Tubular						
Epithelium	2	1		1		
LARYNX (NO. EXAMINED)	(5)			(5)		
LIVER (NO. EXAMINED)	(5)	(5)	(5)	(5)		
Hypertrophy				5		
Inflammation, Chronic, Focal		2	2	4		
LUNG (NO. EXAMINED)	(5)	(5)	(5)	(5)		
Alveolar Epithelium,						
Hyperplasia						
Alveolar Histiocytosis						
Alveoli, Inflammatory Cell						
Infiltration, Focal			2			
Alveoli, Inflammatory Cell						
Infiltration, Multifocal				5		
Interstitium, Fibrosis						
Subpleural Lymphocytes						
NASAL TURB LEVEL I (NO.						
EXAMINED)	(5)	(5)	(5)	(5)		
Respiratory Epithelium,						
Hyperplasia				4		
Respiratory Epithelium,						
Inflammation		3	2			
NASAL TURB LEVEL II (NO.						
EXAMINED)	(5)	(5)	(5)	(5)		
Olfactory Epithelial						
Degeneration			2	5		

SUMMARY INCIDENCE TABLE

08002
Two-Week Sacrifice
Female Rat

	GROUP 1	GROUP 2	GROUP 3	GROUP 4		
NASAL TURB LEVEL III (NO.						
EXAMINED)	(5)	(5)	(5)	(5)		
Olfactory Epithelial						
Degeneration			4	5		
NASAL TURB LEVEL IV (NO.						
EXAMINED)	(5)	(5)	(5)	(5)		
Nasopharyngeal Duct, Goblet						
Cell Hyperplasia/Hypertrophy			4	5		
Olfactory Epithelial						
Degeneration			4	4		
SPLEEN (NO. EXAMINED)	(5)			(5)		
TRACHEA (NO. EXAMINED)	(5)			(5)		

HISTOPATHOLOGY INCIDENCE TABLE

08002
Two-Week Sacrifice
Male Rat

	GROUP 1						GROUP 2						GROUP 3							
ANIMAL	101	102	103	104	105		106	107	108	109	110		111	112	113	114	115			
ADRENAL	X	X	X	X	X															
HEART	X			X	X		X													
Cardiomyopathy		1	1																	
KIDNEY																				
Nephroblastoma																				
Hyaline Droplet, Tubular																				
Epithelium							2	2	2	2	2		2	2	2	2	2			
Infiltrate, Mononuclear Cells													1	1						
Mineralization, Cortical	2	1	1	1	1		1	1		1	2		1	1	1	1	1			
Mineralization, Medullary	1	1	1				1	1	2	1	1		1		1	1	1			
Regeneration, Tubular																				
Epithelium		1						1	1											
LARYNX	X	X	X	X	X															
LIVER	X		X		X															
Hypertrophy							1	1	1	1	1		1	1	1	1	1			
Inflammation, Chronic, Focal		1		1									1	1						
LUNG				X	X			X	X											
Alveolar Epithelium,																				
Hyperplasia																				
Alveolar Histiocytosis										1							1			
Alveoli, Inflammatory Cell																				
Infiltration, Focal														1						
Alveoli, Inflammatory Cell																				
Infiltration, Multifocal																1				
Interstitium, Fibrosis		1																		
Subpleural Lymphocytes	1		1				1				1		1	1	1	1				
NASAL TURB LEVEL I	X	X	X	X	X			X	X	X	X		X				X			
Respiratory Epithelium,																				
Hyperplasia														1						
Respiratory Epithelium,																				
Inflammation							1								1	1				

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Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
n=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

08002
Two-Week Sacrifice
Male Rat

	GROUP 1						GROUP 2						GROUP 3							
ANIMAL	101	102	103	104	105		106	107	108	109	110		111	112	113	114	115			
NASAL TURB LEVEL II	X	X	X	X	X		X	X	X	X	X			X		X	X			
Olfactory Epithelial																				
Degeneration													1		1					
NASAL TURB LEVEL III	X	X	X	X	X		X	X	X	X	X		X	X	X					
Olfactory Epithelial																				
Degeneration																1	1			
NASAL TURB LEVEL IV	X	X	X	X	X		X	X	X	X	X									
Nasopharyngeal Duct, Goblet																				
Cell Hyperplasia/Hypertrophy													2	2	2	2	2			
Olfactory Epithelial																				
Degeneration																				
SPLEEN	X	X	X	X	X															
TRACHEA	X	X	X	X	X															

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P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

08002
Two-Week Sacrifice
Male Rat

	GROUP 4				
ANIMAL	116	117	118	119	120
ADRENAL	X	X	X	X	X
HEART	X	X	X		
Cardiomyopathy				1	1
KIDNEY					
Nephroblastoma					
Hyaline Droplet, Tubular Epithelium	2	2	2	2	2
Infiltrate, Mononuclear Cells					
Mineralization, Cortical	2	2	1	2	1
Mineralization, Medullary	1	1	1	1	
Regeneration, Tubular Epithelium	1				
LARYNX	X	X	X	X	X
LIVER					
Hypertrophy	1	1	2	1	1
Inflammation, Chronic, Focal			1		
LUNG					
Alveolar Epithelium, Hyperplasia		1	1	1	
Alveolar Histiocytosis					
Alveoli, Inflammatory Cell Infiltration, Focal					
Alveoli, Inflammatory Cell Infiltration, Multifocal	1	1	2	2	1
Interstitium, Fibrosis					
Subpleural Lymphocytes		2	2		
NASAL TURB LEVEL I					
Respiratory Epithelium, Hyperplasia		1	1	1	1
Respiratory Epithelium, Inflammation					

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P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

08002
Two-Week Sacrifice
Male Rat

	GROUP 4				
ANIMAL	116	117	118	119	120
NASAL TURB LEVEL II		X			
Olfactory Epithelial					
Degeneration	2		1	1	1
NASAL TURB LEVEL III					
Olfactory Epithelial					
Degeneration	2	3	2	2	1
NASAL TURB LEVEL IV					
Nasopharyngeal Duct, Goblet					
Cell Hyperplasia/Hypertrophy	2	2	2	2	2
Olfactory Epithelial					
Degeneration	2	3	2	2	1
SPLEEN	X	X	X	X	X
TRACHEA	X	X	X	X	X

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1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

08002
Two-Week Sacrifice
Female Rat

	GROUP 1						GROUP 2						GROUP 3							
ANIMAL	201	202	203	204	205		206	207	208	209	210		211	212	213	214	215			
ADRENAL	X	X	X	X	X															
HEART	X	X	X	X	X															
Cardiomyopathy																				
KIDNEY																				
Nephroblastoma								P												
Hyaline Droplet, Tubular Epithelium																				
Infiltrate, Mononuclear Cells																				
Mineralization, Cortical	1	1	1	1	1					1	1		1	1	1	1	1			
Mineralization, Medullary			1	1	1		1	1	1	1	1		1	1	1	1	1			
Regeneration, Tubular Epithelium	1	1							1											
LARYNX	X	X	X	X	X															
LIVER	X	X	X	X	X		X			X	X		X	X			X			
Hypertrophy																				
Inflammation, Chronic, Focal								1	1						1	1				
LUNG	X	X	X	X	X		X	X	X	X	X			X	X		X			
Alveolar Epithelium, Hyperplasia																				
Alveolar Histiocytosis																				
Alveoli, Inflammatory Cell Infiltration, Focal													1			1				
Alveoli, Inflammatory Cell Infiltration, Multifocal																				
Interstitium, Fibrosis																				
Subpleural Lymphocytes																				
NASAL TURB LEVEL I	X	X	X	X	X					X	X		X		X		X			
Respiratory Epithelium, Hyperplasia																				
Respiratory Epithelium, Inflammation							1	1	1					1		1				

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P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

08002
Two-Week Sacrifice
Female Rat

	GROUP 1						GROUP 2						GROUP 3						
ANIMAL	201	202	203	204	205		206	207	208	209	210		211	212	213	214	215		
NASAL TURB LEVEL II	X	X	X	X	X		X	X	X	X	X			X	X	X			
Olfactory Epithelial																			
Degeneration													1				1		
NASAL TURB LEVEL III	X	X	X	X	X		X	X	X	X	X					X			
Olfactory Epithelial																			
Degeneration													1	1	1		1		
NASAL TURB LEVEL IV	X	X	X	X	X		X	X	X	X	X								
Nasopharyngeal Duct, Goblet																			
Cell Hyperplasia/Hypertrophy														2	2	2	2		
Olfactory Epithelial																			
Degeneration													1	1	1		1		
SPLEEN	X	X	X	X	X														
TRACHEA	X	X	X	X	X														

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1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

08002
Two-Week Sacrifice
Female Rat

	GROUP 4				
ANIMAL	216	217	218	219	220
ADRENAL	X	X	X	X	X
HEART		X		X	X
Cardiomyopathy	1		1		
KIDNEY					
Nephroblastoma					
Hyaline Droplet, Tubular Epithelium					
Infiltrate, Mononuclear Cells					
Mineralization, Cortical	1	1	1	1	1
Mineralization, Medullary	1	1	1	2	1
Regeneration, Tubular Epithelium				1	
LARYNX	X	X	X	X	X
LIVER					
Hypertrophy	1	1	1	1	1
Inflammation, Chronic, Focal	1	1	1		1
LUNG					
Alveolar Epithelium, Hyperplasia					
Alveolar Histiocytosis					
Alveoli, Inflammatory Cell Infiltration, Focal					
Alveoli, Inflammatory Cell Infiltration, Multifocal	1	2	1	1	1
Interstitium, Fibrosis					
Subpleural Lymphocytes					
NASAL TURB LEVEL I					X
Respiratory Epithelium, Hyperplasia	1	1	1	1	
Respiratory Epithelium, Inflammation					

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Key : X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

08002
Two-Week Sacrifice
Female Rat

	GROUP 4				
ANIMAL	216	217	218	219	220
NASAL TURB LEVEL II					
Olfactory Epithelial					
Degeneration	1	1	1	1	1
NASAL TURB LEVEL III					
Olfactory Epithelial					
Degeneration	1	2	1	2	1
NASAL TURB LEVEL IV					
Nasopharyngeal Duct, Goblet					
Cell Hyperplasia/Hypertrophy	2	2	2	2	2
Olfactory Epithelial					
Degeneration	1	1		1	1
SPLEEN	X	X	X	X	X
TRACHEA	X	X	X	X	X

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Key : I=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=unschedaled sac./death

08002
Two-Week Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rat Sex: Males Group Identification: 4

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
118	LIVER	Hernia, 5x3x2mm	No Correlating Lesion
	LUNG	Focus(Foci), red, 2x2mm on the caudal lobe	Alveoli, Inflammatory Cell Infiltration, Multifocal
	NASOPHARYNGEAL TISSUES	Discoloration, red, dry	No Comment Required
119	NASOPHARYNGEAL TISSUES	Discoloration, red, dry	No Comment Required
120	NASOPHARYNGEAL TISSUES	Discoloration, red, dry	No Comment Required

08002
Two-Week Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rat Sex: Females Group Identification: 2

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
207	KIDNEY	Right, mass, pale, 12x11x10mm, cut surface firm and pale	Nephroblastoma

08002
Two-Week Sacrifice

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

Species: Rat Sex: Females Group Identification: 4

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
216	LUNG	Focus(Foci), red, 1mm, on caudal lobe	Alveoli, Inflammatory Cell Infiltration, Multifocal
217	LUNG	Focus(Foci), several 1mm pale foci	Alveoli, Inflammatory Cell Infiltration, Multifocal
	NASOPHARYNGEAL TISSUES	Discoloration, red, dry	No Comment Required
220	NASOPHARYNGEAL TISSUES	Discoloration, red, dry	No Comment Required

APPENDIX C. *IN VIVO* BONE MARROW MICRONUCLEUS GENOTOXICITY ASSAY FOR TWO-WEEK INHALATION TOXICITY STUDY OF FT JET FUEL IN RATS

Sponsor: Naval Health Research Center
Environmental Health Effects Laboratory
2729 R St., Bldg 837, Area B
Wright-Patterson AFB, OH 45433-5707

Testing Facility: The Hamner Institutes for Health Sciences
6 Davis Drive
PO Box 12137
Research Triangle Park, NC 27709

Test Site: BioReliance Corporation
9630 Medical Center Drive
Rockville, MD 20850

REVIEW AND APPROVAL

Report prepared by: _____ Date 3/29/2010
Pu Gao, M.D., Research Investigator

Report Reviewed by: _____ Date 3/29/2010
Sheela Sharma, Ph.D., Senior Research Investigator

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GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

This genotoxicity assay portion of Protocol 08002 was conducted in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency, 40 CFR Part 792, except that some data were not recorded according to 792.130(e).

PRINCIPAL INVESTIGATOR:  Date 3/29/2010

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SUMMARY

The genotoxicity of FT Jet fuel in a two-week inhalation toxicity study in F344 rats was investigated in a short-term mutagenicity assay, the mammalian erythrocyte micronucleus (MN) test. Animals were exposed to three concentrations of FT jet fuel, 500 mg/m^3, 1000 mg/m^3 and 2000 mg/m^3 or clean air only (air control group) for 6 hours/day, 5 days per week over a period of two weeks. Immediately after the last exposure, rats were euthanized, and bone marrow cells were collected and processed for micronuclei scoring. The frequency of micronucleated cells were observed by counting 2000 PCEs (polychromatic erythrocytes) per animal. The ratio of PCE to NCE (normochromatic erythrocytes) was used as the indicator of toxicity.

The results indicate that, under the experimental conditions, animals exposed to the low, intermediate, and high concentrations FT Jet fuel, showed no significant dose-related increase in the number of micronucleated PCEs, nor a significant increase in the micronucleated cell frequency in the male or female F344 rat polychromatic erythrocyte system. Therefore, FT Jet fuel is not considered mutagenic in the *in vivo* rat bone marrow cell micronucleus assay.

INTRODUCTION

The *in vivo* mammalian erythrocytes micronucleus (MN) test, which detects the damage of chromosome or mitotic apparatus caused by chemicals, is used to examine the chromosome-damaging effect of the test agent (Schmid, 1975). The damage of chromosome or mitotic apparatus caused by a clastogenic chemical in polychromatic erythrocyte (PCE) stem cells of treated animals is detected in this assay. Micronuclei are believed to be formed from chromosomes or chromosome fragments left behind during anaphase. After telophase, these fragments may not be included in the nuclei of the daughter cells and form single or multiple micronuclei in the cytoplasm. Thus the micronucleus test can serve as a rapid screen for clastogenic agents and test articles that interfere with normal mitotic cell division, affecting spindle fiber function or formation. The assay is based on the increase in the frequency of micronucleated PCEs in bone marrow of the treated animals.

In this study, Fischer-344 rats were exposed by inhalation to an aerosol/vapor mixture of FT jet fuel. Exposures were conducted for 6 hours/day, 5 days/week, for 2 weeks to 3 concentrations of FT jet fuel (500, 1000, and 2000 mg/m^3) and an air control. At the end of exposures, animals were necropsied, and bone marrow extracted from the femur for the MN assay. To check the responsiveness of the test animals, separate sets of F-344 rats were administered a known inducer of micronuclei, cyclophosphamide (CP). CP was dissolved in saline and administered intraperitoneally (ip) to five rats/sex. Saline alone was administered ip to five rats/sex as a vehicle control. The MN control animals were necropsied and the bone marrow extracted for the MN assay. The assay was conducted according to the U.S. EPA Health Effects Tests Guideline OPPTS 870.5395 (1998) and OECD Guideline for the Testing of Chemicals, 474, Mammalian Erythrocytes Micronucleus Test (1997).

STUDY DESIGN

Group	Exposure Level	Number of Animals	
	mg/m^3	Males	Females
Control	0	5	5
Low	500	5	5
Intermediate	1000	5	5
High	2000	5	5
Micronuclei Neg.control	NA	5	5
Micronuclei Pos. control	NA	5	5
Total		30	30

MATERIALS AND METHODS

Animals

Albino Rats: Fischer (CDF®) [F344/DuCrl], approximately 8 weeks-old, were purchased from Charles River Laboratories, Kingston, NY. Upon receipt, animals were acclimated in the Hamner animal facility for approximately two weeks. The experimental animals were identified by ear tag, each with a unique number. Food (NIH-07) and reversed osmosis water were provided *ad libitum* except during inhalation exposure.

Test Chemicals

- FT jet fuel (CAS # 437986-20-4) was provided by sponsor.
- Cyclophosphamide monohydrate (CP) (CAS # 6055-19-2) was obtained (catalog number C7397, Sigma-Aldrich, St. Louis, MO).

Animal Treatment

The test substance, FT jet fuel was administered as an aerosol/vapor combination. Animals were exposed to three concentrations of FT jet fuel, low (500 mg/m^3), intermediate (1000 mg/m^3) and high (2000 mg/m^3) or clean air only (air control group) for six hours/day, five days per week over a period of two weeks. Immediately after the last exposure, rats were euthanized and necropsied (March 7, 2008), and bone marrow cells were collected and processed as described in the preparation of slides. For the MN control groups, physiological saline and CP dissolved in physiological saline (at 40 mg/kg) were administered IP to five negative and five positive controls, respectively, for both male and female rats (March 5, 2008). Animals were observed

10 minutes, 30 minutes and 24 hours after IP injection for toxicity and none was observed. MN control groups were euthanized and bone marrow extracted (March 6, 2008).

Preparation of Slides and Microscopic Observation

Rats were euthanized by intraperitoneal injection of sodium pentobarbital and bone marrow cells were collected by flushing the femur with a mixture of one percent sodium citrate and fetal bovine serum (70:30). After centrifugation, the pellet was re-suspended and bone marrow cell smears were made on duplicate slides (March 6, 2008). One set of slides were stained by May-Grunewald / Giemsa stain (March 11 and 14, 2008). For each slide, two hundred erythrocytes were randomly counted (between March 24 and May 8, 2008) and the number of polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) were recorded separately. Micronucleated polychromatic erythrocytes (MPCEs) in 2000 PCEs were observed randomly and recorded. The number of PCEs, NCEs and MPCEs were recorded and scored blindly. The micronuclei usually appear as densely stained small round bodies in the cytoplasm of the PCEs. The general size of the micronuclei is approximately 1/20 to 1/5 of diameter of the PCEs. Images 1 and 2 show the NCEs and micronuclei containing PCEs (MPCEs) in positive control CP slides (May-Grunewald / Giemsa staining). The results are expressed as percent of micronucleated PCEs in total PCEs and statistically analyzed with ANOVA, Student t-test, and a p value of <0.05 is considered as significant. Raw data analyzed in this report are found in Appendices I – III, while unused data are preserved in Appendix IV.

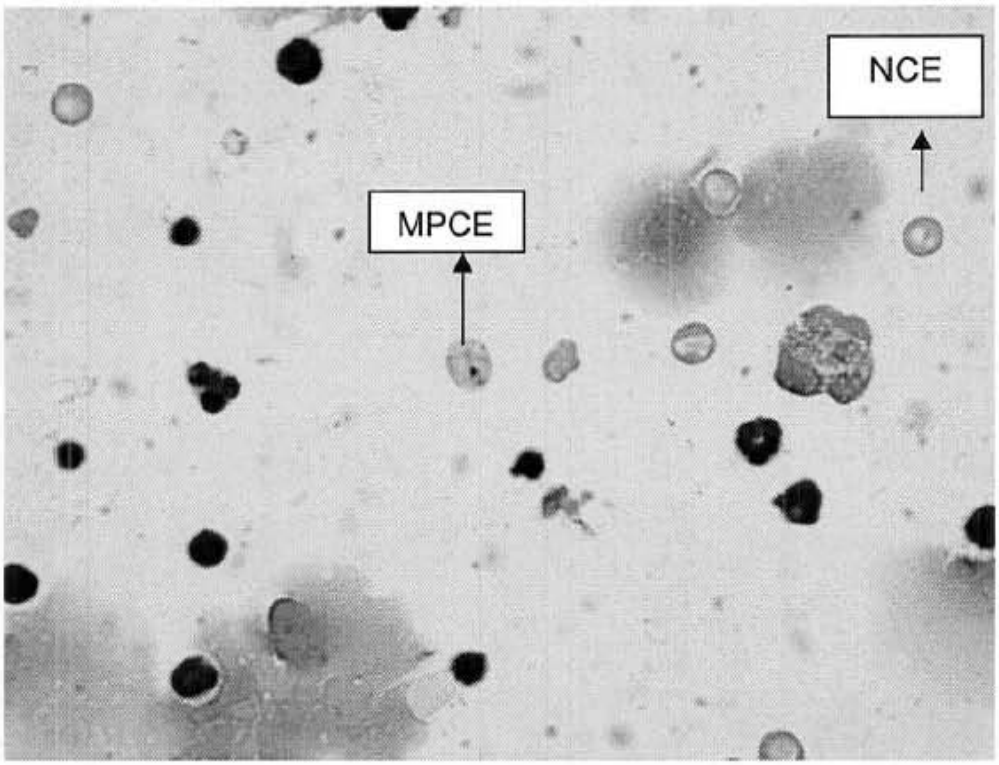

Image 1 Micronucleated PCE and NCE in Bone Marrow Cells (Positive Control)

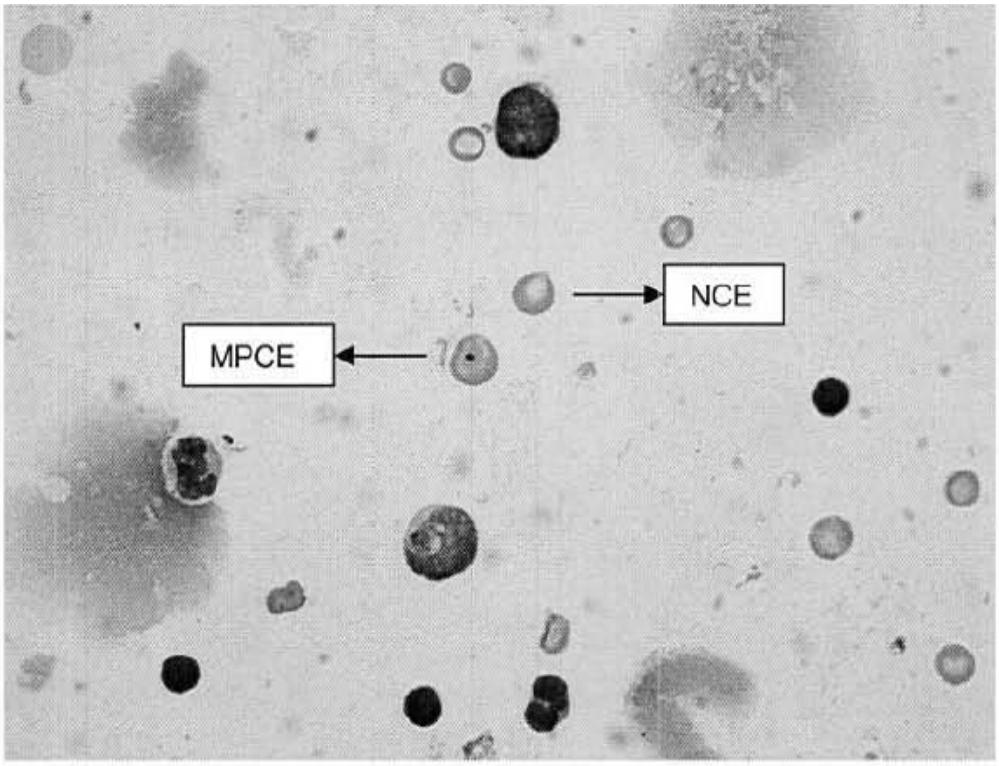

Image 2 Micronucleated PCE and NCE in Bone Marrow Cells (Positive Control)

After an initial assessment of the MN data from the May-Grunewald / Giemsa stained slides, it was determined that numbers of MPCE in air control animals (males and females) was not normal based on historical data from our laboratory, and the range of 0.1 to·0.3 percent reported in the literature (Heddle *et al.*, 1983; Mavournin *et al.*, 1990). Due to key personnel being on extended leave out of the country, it was decided to contract with an outside laboratory, BioReliance Corporation, Rockville, MD, to provide an independent assessment of MPCEs (see Appendix V). Stained slides were sent to BioReliance for counting. However, BioReliance uses a different stain for their MN procedure, so a portion of the duplicate set of unstained slides was sent to BioReliance, where the slides were stained with acridine orange and counted using the procedure described in their report (Appendix VI). MPCE data from BioReliance were used for the assessment of genotoxicity in the jet fuel exposed animals, while MPCE data from The Hamner Institutes were used to assess the MN (CP) control animals.

RESULTS

Toxicity of FT Jet Fuel

The test agent toxicity is determined from the ratio of PCE to NCE. The normal ratio of PCE to NCE in rat bone marrow cells is usually 0.5 to 0.6 (Gollapudi and McFadden, 1995) or the ratio of treated animals should not be less than 20 percent of control animals (MacGregor, 1987; OECD, 1997). Results from the BioReliance assessment showed that the ratio of PCE to NCE was in the normal range for all groups tested including control. Although the PCE to NCE ratio and percentage of PCEs in total erythrocytes was lower than the control group for the male low concentration and female low and high concentrations, statistical analysis of the data showed that toxicity only appeared in the female high concentration (p-value = 0.04). Therefore, FT jet fuel was not toxic to F344 rat bone marrow cells in most of doses exposed (Table 1). The BioReliance report also used the ratio of PCE to total erythrocytes (EC). They determined that a reduction of PCEs/ECs of up to 16 percent was observed in the exposed animals versus air controls. However, there was not a dose-dependent relationship; hence bone marrow toxicity was not indicated.

Table 1. Summary of Toxicity Data in Rat Bone Marrow Cells after Exposure to FT Jet Fuel

Group	Treatment mg/m^3	PCE/NCE Ratio	*p* Value	% of PCE In total erythrocytes	*p* Value
Male					
Air Ctrl	Clean Air	0.77 ± 0.23		42.72 ±7.02	
FT Low	500	0.56 ± 0.08	0.12	35.94±3.48	0.10
FT Intermediate	1000	0.78 ± 0.25	0.95	42.94±6.99	0.96
FT High	2000	0.70 ± 0.12	0.56	40.90±4.10	0.63
Female					
Air Ctrl	Clean Air	0.73 ± 0.13		42.02±4.38	
FT Low	500	0.61 ± 0.13	0.56	37.70±4.83	0.63
FT Intermediate	1000	0.69 ± 0.16	0.63	40.20±6.15	0.61
FT High	2000	0.57 ± 0.06	0.04	36.20±2.27	0.04

Micronuclei Induction

The results of the effect of FT jet fuel on the bone marrow cell micronucleus induction are presented in Table 2. The background frequencies of micronucleated PCEs are usually in the range of 0.1 to 0.3 percent (Heddle *et al.*, 1983; Mavournin *et al.*, 1990). In this study, the background (air control groups) frequency of micronucleated PCE ranged from 0.02 to 0.03

percent, which is in the normal range. There was no significant increase in the number of micronucleated PCEs in all treatment groups, whether male or female, compared to air control groups. Similarly, the micronuclei frequency in the negative control for CP (saline control) was 0.11 percent, whereas, the positive control (CP) at 40 mg/kg, induced micronuclei frequency by 5- to 9-fold in both male and female rats. Therefore, this micronuclei induction study with FT jet fuel is considered valid. The analysis by BioReliance, in which they used Kastenbaum-Bowman Tables to determine significance, also concluded that there was no significant incidence in MPCE in the bone marrow of rats exposed to FT jet fuel.

Table 2. Data on Micronuclei Analysis in F-344 Rat Bone Marrow after Exposure to FT Jet Fuel

Group	Treatment	% of MPCE in 2000 PCE	*p* Value
Male			
Air Ctrl	Clean Air	0.02 ± 0.03	
FT-Low	500 mg/m^3	0.00 ± 0.00	
FT-Intermediate	1000 mg/m^3	0.03 ± 0.03	0.58
FT-High	2000 mg/m^3	0.03 ± 0.03	0.58
Negative Control	Saline	0.13 ± 0.07	
Positive Control	CP, 40 mg/kg	0.62 ± 0.13	
Female			
Air Ctrl	Clean Air	0.03 ± 0.03	
FT-Low	500 mg/m^3	0.02 ± 0.03	0.58
FT-Intermediate	1000 mg/m^3	0.01 ± 0.02	0.24
FT-High	2000 mg/m^3	0.02 ± 0.03	0.58
Negative Control	Saline	0.09 ± 0.09	
Positive Control	CP, 40 mg/kg	0.82 ± 0.18	

See appendix I for individual animal data for FT jet fuel study groups
See appendix II for individual animal data for Negative and Positive Control groups

DISCUSSION

The FT jet fuel is a liquid hydrocarbon compound mainly developed as a replacement of petroleum derived JP-8 jet fuel for military use by U.S. military. Jet fuels contain complex mixtures of aliphatic, aromatic and other substituted naphthalene hydrocarbon compounds. There is a wide range of information on the human health effects of exposure to jet fuels including JP-8 with respect to cardiovascular, reproductive system, respiratory tract, immune, neuronal, renal and carcinogenicity profiles (NRC, 2003). There are very limited reliable data regarding the genotoxicity of JP-8 and other jet fuels. Even though most of the *in vitro* data on mutagenicity were negative (McKee *et al.*, 1989; Nessel *et al.*, 1999), there were reports of

significant increases in DNA single strand breaks in mammalian cells exposed *in vitro* and *in vivo* to jet fuels including JP-8 (Grant *et al.*, 2000; 2001). However, single or repeated dermal exposures of mice with jet fuels JP-8 and Jet A failed to cause any statistically significant increase in the incidence of MN in PCEs (Vijayalaxmi *et al.*, 2006). In an earlier study, the same authors have observed a significant increase in MN when the mice were treated with one dose of the same jet fuels (Vijayalaxmi *et al.*, 2004). However, our studies with repeated inhalation exposures of two weeks to low, intermediate and high concentrations of FT failed to induce MN both in male and female rats.

CONCLUSION

The present MN study indicates that, under the experimental conditions, F-344 rats (male and female) treated with FT jet fuel at concentrations of 500, 1000, and 2000 mg/m^3 for two weeks showed no significant increase in the number of micronucleated PCEs. Therefore, FT jet fuel should not be considered mutagenic in the *in vivo* rat bone marrow micronucleus assay.

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APPENDIX I. RAW DATA OF MICRONUCLEI SCORING IN F344 RATS AFTER EXPOSURE TO FT JET FUEL (PROTOCOL #08002) (DATA FROM BIORELIANCE CORPORATION)

Group	Animal Number	PCE/ 1000 Erythrocytes	NCE/ 1000 Erythrocytes	PCE/NCE Ratio	% of PCE/ Total Erythrocytes	# of MPCE /2000 PCEs	% MPCE / Total PCE
Male							
Ctrl.	101	402	598	0.7	40.20	0	0.00
	102	489	511	1.0	48.90	1	0.05
	103	359	641	0.6	35.90	0	0.00
	104	514	486	1.1	51.40	0	0.00
	105	373	627	0.6	37.20	1	0.05
	Mean	**427.2**	**572.8**	**0.77**	**42.72**	**0.40**	**0.02**
	SD	**70.15**	**70.15**	**0.23**	**7.02**	**0.55**	**0.03**
500 mg/m^3	106	378	622	0.6	37.80	0	0.00
	107	372	628	0.6	37.20	0	0.00
	108	388	612	0.6	38.80	0	0.00
	109	300	700	0.4	30.00	0	0.00
	110	359	641	0.6	35.90	0	0.00
	Mean	**359.40**	**640.60**	**0.56**	**35.94**	**0.00**	**0.000**
	SD	**34.83**	**34.83**	**0.08**	**3.48**	**0.00**	**0.00**
	***P*- value**			**0.12**	**0.10**	**0.18**	**0.18**

Group	Animal Number	PCE/ 1000 Erythrocytes	NCE/ 1000 Erythrocytes	PCE/NCE Ratio	% of PCE/ Total Erythrocytes	# of MPCE /2000 PCEs	% MPCE / Total PCE
1000 mg/m^3	111	549	451	1.2	54.90	1	0.05
	112	411	589	0.7	41.10	1	0.05
	113	416	584	0.7	41.60	0	0.00
	114	365	635	0.6	36.50	0	0.00
	115	406	594	0.7	40.60	1	0.05
	Mean	**429.40**	**570.60**	**0.78**	**42.94**	**0.60**	**0.03**
	SD	**69.85**	**69.85**	**0.25**	**6.99**	**0.55**	**0.03**
	***P*- value**			**0.95**	**0.96**	**0.58**	**0.58**
2000 mg/m^3	116	422	578	0.7	42.20	0	0.00
	117	468	532	0.9	46.80	0	0.00
	118	370	630	0.6	36.90	1	0.05
	119	415	585	0.7	41.40	1	0.05
	120	371	629	0.6	37.10	1	0.05
	Mean	**408.80**	**591.20**	**0.70**	**40.90**	**0.60**	**0.03**
	SD	**40.98**	**40.98**	**0.12**	**4.10**	**0.55**	**0.03**
	***P*- value**			**0.56**	**0.63**	**0.58**	**0.58**

Group	Animal Number	PCE/ 1000 Erythrocytes	NCE/ 1000 Erythrocytes	PCE/NCE Ratio	% of PCE/ Total Erythrocytes	# of MPCE /2000 PCEs	% MPCE / Total PCE
Female							
Ctrl.	201	397	603	0.7	39.70	1	0.05
	202	437	563	0.8	43.70	0	0.00
	203	355	645	0.6	35.50	1	0.05
	204	452	548	0.8	45.10	1	0.05
	205	461	539	0.9	46.10	0	0.00
	Mean	**420.20**	**579.80**	**0.73**	**42.02**	**0.60**	**0.03**
	SD	**43.83**	**43.83**	**0.13**	**4.38**	**0.55**	**0.03**
500 mg/m^3	206	380	620	0.6	37.90	1	0.05
	207	453	547	0.8	45.30	0	0.00
	208	369	631	0.6	36.80	1	0.05
	209	319	681	0.5	31.90	0	0.00
	210	366	634	0.6	36.60	0	0.00
	Mean	**377.00**	**623.00**	**0.61**	**37.70**	**0.40**	**0.02**
	SD	**48.34**	**48.34**	**0.13**	**4.83**	**0.55**	**0.03**
	***p*-value**			**0.56**	**0.63**	**0.58**	**0.58**

Group	Animal Number	PCE/ 1000 Erythrocytes	NCE/ 1000 Erythrocytes	PCE/NCE Ratio	% of PCE/ Total Erythrocytes	# of MPCE /2000 PCEs	% MPCE / Total PCE
1000 mg/m^3	211	307	693	0.4	30.70	0	0.00
	212	465	535	0.9	46.50	0	0.00
	213	442	558	0.8	44.20	0	0.00
	214	382	618	0.6	38.20	0	0.00
	215	414	586	0.7	41.40	1	0.05
	Mean	**402.00**	**598.00**	**0.69**	**40.20**	**0.20**	**0.01**
	SD	**61.52**	**61.52**	**0.16**	**6.15**	**0.45**	**0.02**
	***p*-value**			**0.63**	**0.61**	**0.24**	**0.24**
2000 mg/m^3	216	352	648	0.5	35.20	0	0.00
	217	329	671	0.5	32.90	0	0.00
	218	389	611	0.6	38.90	1	0.05
	219	368	632	0.6	36.80	0	0.00
	220	372	628	0.6	37.20	1	0.05
	Mean	**362.00**	**638.00**	**0.57**	**36.20**	**0.40**	**0.02**
	SD	**22.66**	**22.66**	**0.06**	**2.27**	**0.55**	**0.03**
	***p*-value**			**0.04**	**0.04**	**0.58**	**0.58**

APPENDIX II. RAW DATA OF MICRONUCLEI SCORING IN NEGATIVE AND POSITIVE CONTROL F344 RATS (PROTOCOL #08002) (DATA FROM THE HAMNER INSTITUTES)

Animal Number	Group	Total Erythrocytes	NCE /1000 Erthrocytes	PCE /1000 Erythrocytes	PCE/NCE Ratio	% of PCE /total Erythrocytes	Total PCE	MPCE	% MPCE
Male									
121	**Saline**	335	160	175	1.1	52.24	2088	2	0.10
122		258	150	108	0.7	41.86	2070	5	0.24
123		221	113	108	1.0	48.87	2010	2	0.10
124		338	136	202	1.5	59.76	2084	3	0.14
125		236	144	92	0.6	38.98	2026	1	0.05
Mean		**277.60**	**140.60**	**137.00**	**0.98**	**48.34**	**2055.60**	**2.60**	**0.13**
SD		**55.37**	**17.74**	**48.41**	**0.34**	**8.30**	**35.42**	**1.52**	**0.07**
126	**Pos. Ctrl.**	323	211	112	0.5	34.67	2082	10	0.48
127		240	145	95	0.7	39.58	2014	10	0.50
128		278	168	110	0.7	39.57	2075	14	0.67
129		272	179	93	0.5	34.19	2027	16	0.79
130		287	175	112	0.6	39.02	2037	13	0.64
Mean		**280.00**	**175.60**	**104.40**	**0.60**	**37.41**	**2047**	**12.60**	**0.62**
SD		**29.86**	**23.77**	**9.56**	**0.07**	**2.73**	**29.99**	**2.61**	**0.13**

Animal Number	Group	Total Erythrocytes	NCE /200 Erthrocytes	PCE /200 Erythrocytes	PCE/NCE Ratio	% of PCE /total Erythrocytes	Total PCE	MPCE	% MPCE
Female									
221	**Saline**	235	143	92	0.6	39.15	2038	5	0.25
222		245	150	95	0.6	38.78	2041	1	0.05
223		257	149	108	0.7	42.02	2024	0	0.00
224		254	153	101	0.7	39.76	2032	2	0.10
225		211	87	124	1.4	58.77	2038	1	0.05
Mean		**205.31**	**117.63**	**88.26**	**0.69**	**36.87**	**1700.50**	**1.93**	**0.09**
SD		**87.54**	**52.30**	**40.21**	**0.43**	**18.38**	**818.40**	**1.75**	**0.09**
226	**Pos. Ctrl.**	292	182	108	0.6	37.11	2041	15	0.73
227		429	273	156	0.6	36.36	2027	20	0.99
228		365	211	154	0.7	42.19	2037	21	1.03
229		376	250	126	0.5	33.51	2003	16	0.80
230		226	143	83	0.6	36.73	2038	11	0.54
Mean		**349.00**	**219.25**	**129.75**	**0.60**	**37.20**	**2026.25**	**17.00**	**0.82**
SD		**86.63**	**56.91**	**34.04**	**0.10**	**3.63**	**16.28**	**4.50**	**0.18**

APPENDIX III. RAW DATA OF MICRONUCLEI SCORING IN NEGATIVE AND POSITIVE CONTROL F344 RATS (PROTOCOL #08002) (DATA FROM BIORELIANCE CORP)

Animal Number	Group	Total Erythrocytes	NCE /1000 Erthrocytes	PCE /1000 Erythrocytes	PCE/NCE Ratio	% of PCE /total Erythrocytes	Total PCE	MPCE	% MPCE
122 (M)	Saline	1000	517	483	0.93	51.7	2000	1	0.05
223 (F)	Saline	1000	534	466	0.87	53.4	2000	0	0.00
129 (M)	CP	1000	723	277	0.38	72.3	2000	21	1.05
226 (F)	CP	1000	703	297	0.42	70.2	2000	20	1.00

APPENDIX IV. RAW DATA OF MICRONUCLEI SCORING IN F344 RAT BONE MARROW AFTER EXPOSURE TO FT JET FUEL (PROTOCOL #08002) (UNUSED DATA FROM THE HAMNER INSTITUTE)

Animal #	Group	# NCE	# PCE	Total Erythrocytes	PCE/NCE	% of PCE in erythrocytes	Total PCE	# of MPCE	% of MPCE
Male									
101	**Control**	140	70	210	0.5	33.33	2068	23	1.11
102		129	106	235	0.8	45.11	2095	8	0.38
103		109	92	201	0.8	45.77	2023	7	0.35
104		92	109	201	1.2	54.23	2006	10	0.50
105		163	77	240	0.5	32.08	2021	17	0.84
106	**500 mg/m^3**	133	80	213	0.6	37.56	2003	1	0.05
107		100	90	190	0.9	47.37	2058	1	0.05
108		159	80	239	0.5	33.47	2028	2	0.10
109		147	75	222	0.5	33.78	2015	3	0.15
110		183	88	271	0.5	32.47	2018	3	0.15
111	**1000 mg/m^3**	125	95	220	0.8	43.18	2032	4	0.20
112		132	74	206	0.6	35.92	2011	4	0.20
113		147	85	232	0.6	36.64	2025	15	0.74
114		133	83	216	0.6	38.43	2067	24	1.16
115		130	99	229	0.8	43.23	2012	22	1.09
116	**2000 mg/m^3**	106	112	218	1.1	51.38	2012	5	0.25
117		102	101	203	1.0	49.75	2064	12	0.58
118		136	99	235	0.7	42.13	2185	23	1.05
119		116	94	210	0.8	44.76	2014	4	0.20
120		153	96	249	0.6	38.55	2003	25	1.25

Animal #	Group	# NCE	# PCE	Total Erythrocytes	PCE/NCE	% of PCE in erythrocytes	Total PCE	# of MPCE	% of MPCE
Female									
201	**Control**	122	105	227	0.9	46.26	2013	24	1.19
202		112	100	212	0.9	47.17	2008	11	0.55
203		139	91	230	0.7	39.57	2021	45	2.23
204		130	85	215	0.7	39.53	2035	8	0.39
205		127	106	233	0.8	45.49	2019	5	0.25
206	**500 mg/m^3**	105	102	207	1.0	49.28	2020	2	0.10
207		117	93	210	0.8	44.29	2024	2	0.10
208		127	83	210	0.7	39.52	2026	5	0.25
209		150	93	243	0.6	38.27	2031	4	0.20
210		112	105	217	0.9	48.39	2034	4	0.20
211	**1000 mg/m^3**	169	75	244	0.4	30.74	2010	4	0.20
212		118	102	220	0.9	46.36	2069	4	0.19
213		163	92	255	0.6	36.08	2007	27	1.35
214		171	68	239	0.4	28.45	2012	3	0.15
215		138	82	220	0.6	37.27	2018	5	0.25
216	**2000 mg/m^3**	134	72	206	0.5	34.95	2024	5	0.25
217		186	61	247	0.3	24.70	2014	13	0.65
218		167	55	222	0.3	24.77	2003	1	0.05
219		154	100	254	0.6	39.37	2011	4	0.20
220		91	126	217	1.4	58.06	2005	2	0.10

APPENDIX V: MEMORANDUM

THE HAMNER INSTITUTES FOR HEALTH SCIENCES
WHERE GREAT MINDS & MEDICINE MEET

MEMORANDUM

TO: Brian A. Wong, Ph.D.
Study Director, Protocol #08002

FROM: Sheela Sharma, Ph.D.
Co-PI

DATE: December 16, 2008

SUBJECT: Micronuclei (MN) Assay in Two-Week Inhalation Study of FT Jet Fuel in Rats (08002)

This memo is to clarify why there was an independent assessment of MN scoring of bone marrow slides from this study. Our initial assessment of data generated from scoring MN in slides of control and jet fuel exposed animals indicated that the numbers of MN (spontaneous induction of MN) in the control animals (males and females) was not normal based on historical data from our laboratory. As the Principal Scientist, Dr. Pu Gao, who could have verified the scores, was away in China, a decision was made to get an independent evaluation of MN by rescoring blinded slides. Therefore, stained and unstained slides from control and treated animals were sent to BioReliance, Rockville, MD for a second evaluation of MN scoring.

BioReliance uses a different stain for the bone marrow slides than we use. They preferred to stain the 08002 unstained bone marrow slides from control and treated animals with the stain, acridine orange and evaluated the newly stained slides for MN scoring.

The BioReliance data will be presented in the final report for Study 08002. The data produced at The Hamner Institutes for Health Sciences will be included in Appendix III of the final report as "unused data" along with this memo.

SS:jg

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APPENDIX VI. BIORELIANCE REPORT

CONTRIBUTING SCIENTIST REPORT

Study Title

Bone Marrow Micronucleus Assessment Following a Two Week Inhalation Toxicity Study of FT Jet Fuel in Rats

In support of The Hamner Institute for Health Sciences Study conducted for Naval Health Research Center (Protocol Number 08002): Two - Week Inhalation Toxicity Study of FT Jet Fuel in Rats

Test Article

FT Jet Fuel with additives

Authors

Ljubica Krsmanovic, PhD.
Kathyayini Divi, M.S.

Study Completion Date

14 September 2009

Test Site

BioReliance Corporation
9630 Medical Center Drive
Rockville, MD 20850, U.S.A

BioReliance Study Number

ACI7NH.129GLP.BTL

Testing Facility

The Hamner Institutes for Health Sciences
Six Davis Drive
PO Box 12137
Research Triangle Park, NC 27709, U.S.A.

Sponsor

Naval Health Research Center
Environmental Health Effects Laboratory
2729 R St., Bldg 837, Area B
Wright-Patterson AFB, OH 45433-5707, U.S.A.

STATEMENT OF COMPLIANCE

Microscopic evaluation of bone marrow smears and analysis of data was performed by BioReliance under the study number ACI7NHJ29GLP.BTL as a part of the Hamner Institutes for Health Sciences Protocol Number 08002, for Naval Health Research Center in compliance with U.S. EPA Good Laboratory Practice Regulations 40 CFR 792.

Ljubica Krsmanovic

Ljubica Krsmanovic, Ph.D.
Principal Investigator
BioReliance Corporation

14 September 2009
Date

Eias Zahalka, Ph.D. MBA
BioReliance Corporation

14 Sept 2009
Date

QUALITY ASSURANCE STATEMENT

BioReliance

Quality Assurance Statement

Study Information

Number: AC17NH.129GLP.BTL

Compliance

Procedures, documentation, equipment and other records were examined in order to assure this portion of the multi site study was performed in accordance with the regulation(s) listed below and conducted according to the client study protocol/statement of work and relevant BioReliance Standard Operating Procedures.

US EPA Good Laboratory Standards 40CFR 792

Inspections

Quality Assurance performed the inspections(s) below for this portion of the multi site study.

Insp. Dates (From/To)		Phase Inspected	To Principal Investigator	To Test Site Management	To Study Director & Facility Management
28-Jul-2008	28-Jul-2008	Observation of Test System	28-Jul-2008	28-Jul-2008	28-Jul-2008
14-Oct-2008	14-Oct-2008	Data and Draft Reporting	15-Oct-2008	15-Oct-2008	14-Sep-2009
09-Sep-2009	10-Sep-2009	Final Reporting	10-Sep-2009	10-Sep-2009	14-Sep-2009

The Final Report for this portion of the multi site study identified above describes the methods and procedures and attests that the reported results accurately reflect the raw data.

E-signature

Test Site Quality Assurance: Jessica Mandaci 14-Sep-2009 3:41 pm GMT

Reason for signature: QA Approval

SUMMARY

The objective of this portion of the study was to evaluate the genotoxic (clastogenic/aneugenic) potential of FT jet fuel following a two-week inhalation exposure in rats. The bone marrow was analyzed for the presence of micronucleated polychromatic erythrocytes (MN-PCEs), which served as biomarker/parameter of genotoxicity. Fischer 344 rats were received, acclimated and exposed to the test article at the testing facility (The Hamner Institutes for Health Sciences). For the micronucleus assessment, femoral bone marrow was collected from five rats/sex exposed either to the control (clean air) or FT jet fuel at low (500 mg/m^3), intermediate (1000 mg/m^3) or high concentrations (2000 mg/m^3). Animals were exposed six hours per day, for five days a week, over a two week period. To ensure the responsiveness of the test subjects, five rats/sex were dosed with saline and an additional five rats/sex were dosed with cyclophosphamide (CP, positive control at 40 mg/kg). The Saline and CP were intraperitoneally administered to the animals 24 hours before the euthanization of inhalation exposed animals. Bone marrow collection and slide preparation were performed at the testing facility by their personnel. Staining of slides, microscopic evaluation and reporting of the results were performed by BioReliance (Test Site). Bone marrow smears from the animals that were exposed to control (clean air) and test article and from one animal/sex that received saline or CP were stained and 2000 polychromatic erythrocytes (PCEs) per each animal were microscopically evaluated for the presence of micronuclei (MN-PCEs). A statistical analysis of data was performed using Kastenbaum-Bowman Tables (binomial distribution, $p \leq 0.05$).

The microscopic evaluation and analysis of data indicated that there was no statistically significant increase in the incidence of MN-PCEs in the bone marrow of male or female rats exposed to FT jet fuel relative to the concurrent and respective controls. Reductions in the PCEs/ECs of up to 16 percent were observed in the male and female FT jet fuel groups relative to the respective controls but not in a dose-dependent manner. The reductions of this magnitude suggest that the test article did not inhibit erythropoiesis.

In conclusion, under the conditions of the study conducted, FT jet fuel at exposure levels up to and including 2000 mg/m^3 did not induce a significant increase in the incidence of MNPCEs in bone marrow of male or female Fischer 344 rats. Therefore, FT jet fuel was concluded to have no clastogenic/aneugenic effect when animals were exposed to inhalation six hours/day, five days a week over a two week period.

MATERIAL AND METHODS

Study Design

The Study design is presented in the following Table:

Group	Group Designation	Exposure Level	Number of Animals	
			Males	Females
1	Control Group: Clean air	0 mg/m^3	5	5
2	FT Jet Fuel: Low exposure	500 mg/m^3	5	5
3	Intermediate Exposure	1000 mg/m^3	5	5
4	High Exposure	2000 mg/m^3	5	5
5/Saline	Negative Control for CP	0 mg/kg	5*	5*
6/Cyclophosphamide monohydrate (CP)	Positive Control	40 mg/kg	5*	5*

*Bone marrow from only one animal/sex was microscopically evaluated for the presence of MN-PCEs.

The study was composed of four groups each consisted of five male and five female Fischer 344 rats; the animals in these groups were designated to receive clean air (Group I), or FT jet fuel at low, intermediate and high exposure (Groups 2, 3 and 4) by inhalation. In addition, five male and five female rats were intraperiotoneally dosed with saline and the other five males and five females were dosed with the positive control. Only the bone marrow from one animal/sex was consequently used for micronucleus assessment.

Fischer 344 rats were received and acclimated at the testing facility. Handling of animals, inhalation procedure and observation of animals following inhalation are presented in the report generated by The Hamner Institutes for Health Sciences.

Bone Marrow Collection and Slide Preparation for Micronucleus Assessment

After completion of the two week exposure period, at the time of necropsy, femoral bone marrow smears (slides) were prepared by The Hamner Institutes for Health Sciences personnel and were shipped to BioReliance. Initially, 44 bone marrow slides, blinded (coded) and stained bluish, were received by BioReliance on 01 July 2008. Upon receipt, the code number ACI7NH, sample 0001, was assigned. However, these slides were not evaluated due incompatibility of the bluish stain with the evaluation procedures. A second set of unstained (coded) slides was shipped by the testing facility and BioReliance received these slides on 23 July 2008. The code number of AC17NH, sample 0002, was assigned to this set of slides. At BioReliance, the slides were stained with Acridine orange on 24 July 2008. Microscopic evaluation of bone marrow smears was performed from 26 July 2008 to 28 July 2008.

Bone Marrow Micronucleus Analysis

Microscopic evaluation of bone marrow smears and analysis of data was performed in accordance with the U.S. EPA Health Effects Test Guidelines, Mammalian Erythrocytes Micronucleus Test, EPA OPPTS 870.5395 (1998).

At BioReliance, prior to scoring, the bone marrow slides were stained with Acridine orange (nucleic acid specific stain). Using a fluorescent microscope and medium magnification (400X; blue excitation filter in the range of 440 to 490 nm and barrier filter combination at 520 nm), an area of acceptable quality was selected such that the cells were well spread and stained. Using oil immersion (1000X), the following cell populations and cell components were evaluated and enumerated:

- Polychromatic erythrocytes (PCEs): PCEs stain orange-red. PCEs are young erythrocytes (early stage of erythropoiesis) and are the target cells for evaluation of the test article c1astogenicity. Two-thousand PCEs per each animal were screened (scored) for the presence of micronuclei resulting in evaluation of a total of 10,000 PCEs per control and test article groups and 2000 PCEs per negative and positive control groups.

- Normochromatic erythrocytes (NCEs): NCEs appear light green in color. NCEs are mature erythrocytes (red blood cells) and are the final cell population formed during erythropoiesis. The number of NCEs and micronucleated NCEs (MN-NCEs) in the field of 1000 total erythrocytes (ECs = PCEs + MN-PCEs + NCEs + MN-NCEs = 1000 ECs) was enumerated for each animal in order to calculate the proportion of polychromatic erythrocytes to total of 1000 erythrocytes. The incidence of MN-NCEs per 2000 PCEs was enumerated for each animal, but the results were not presented in this report or used in analysis of the test article induced genotoxic response since the primary target cells are the young erythrocytes, PCEs.

- Micronuclei (MN): Micronuclei are round, fluorescent green-stained nuclear (chromosome) fragments with sharp contours and diameters commonly 1/20 to 1/5 that of an erythrocyte. Micronuclei may occur in PCEs (MN-PCEs) or NCEs (MN-NCEs).

Slides were evaluated by code numbers, and a key to un-code the slides was sent by the testing facility after completion of evaluation.

EVALUATION OF TEST RESULTS

The incidence of micronucleated polychromatic erythrocytes per 2000 polychromatic erythrocytes for each rat and per 10,000 PCEs per control and test article groups and 2000 PCEs per negative (saline) and positive control groups was determined. A statistical evaluation of data at BioReliance was performed using Kastenbaum-Bowman Tables for significant level of $p \leq 0.05$. All analyses were performed separately for each sex.

In order to quantify the proliferation state of the bone marrow as an indicator of bone marrow toxicity, the proportion of polychromatic erythrocytes to total erythrocytes was determined for each rat and treatment group (PCEs/ECs ratio).

All conclusions were based on scientific judgment. As a guide to interpretation of the data, the following were considered:

- The test article would have been considered to induce a positive response if a dose-responsive increase in the incidence of micronucleated polychromatic erythrocytes in bone marrow or peripheral blood was observed and one or more doses were statistically elevated relative to the control ($p \leq 0.05$, Kastenbaum-Bowman Tables).

- The test article was judged negative because no statistically significant increase in the incidence of micronucleated polychromatic erythrocytes above the concurrent vehicle control values and no evidence of dose response were observed.

- If criteria for either a positive or negative clastogenic response were not met, the results would have been judged as equivocal.

RECORDS AND ARCHIVES

Following the issuance of the final report, all raw data, the protocol, all reports and slides will be forwarded to The Hamner Institutes for storage and archiving as per Section II of the study protocol. Prior to shipping the study-related material for archiving, all study materials will be first copied onto electronic media and the electronic copy will be retained at BioReliance, according to Standard Operating Procedure OPQP3040.

All training records of the personnel involved in the conduct of the study as well as all other facility records will be kept at BioReliance.

DEVIATIONS

No known deviations from the protocol or assay method SOPs occurred during the conduct of the portion of the study conducted at BioReliance.

TESTING AND REGULATORY REQUIREMENTS

This portion of the study was conducted in compliance with U.S. EPA Health Effects Test Guidelines, Mammalian Erythrocytes Micronucleus Test, EPA OPPTS 870.5395 (1998) and the U.S. EPA Good Laboratory Practice Regulations as published in 40 CFR 792.

RESULTS AND DISCUSSION

The results of bone marrow micronucleus analysis are presented in Table 1 (summary data) and Table 2 (individual data).

The results indicate the following:

- Reductions in the PCEs/ECs of up to 16 percent were observed in the male and female FT jet fuel groups relative to the respective controls but not in a dose-dependent manner. The reductions of this magnitude suggest that the test article was bio-available.

- No statistically significant increase in the incidence of micronucleated polychromatic erythrocytes in bone marrow was observed in male or female FT jet fuel groups relative to the respective control groups (clean air).

- The incidence of MN-PCEs in the bone marrow of one male and one female rats dosed with the positive control was significantly increased when compared with the negative control (saline).

CONCLUSION

In conclusion, under the conditions of the study conducted, FT jet fuel at exposure levels up to and including 2000 mg/m^3 did not induce a significant increase in the incidence of MNPCEs in bone marrow of male or female Fischer 344 rats. Therefore, FT jet fuel was concluded to have no clastogenic/aneugenic effect when animals were exposed by inhalation for six hours/day, five days/week over a two week period.

Table 1: Summary of Bone Marrow Micronucleus Analysis after Two Week Exposure of 344 Fischer Rats to FT Jet Fuel

Treatment	Sex	Time (hr)	Number of Animals	PCE/Total Erythrocytes (Mean +/- SD)	Change from Control (%)	Number of MN-PCE/1000 PCE (Mean +/- SD)	Number of MN-PCE/PCE Scored
Clean Air	M	24	5	0.427 ± 0.07	---	0.2 ± 0.27	2 / 10000
	F	24	5	0.420 ± 0.04	---	0.3 ± 0.27	3 / 10000
FT Jet Fuel							
500 mg/m^3	M	24	5	0.359 ± 0.03	-16	0.0 ± 0.00	0 / 10000
	F	24	5	0.377 ± 0.05	-10	0.2 ± 0.27	2 / 10000
1000 mg/m^3	M	24	5	0.429 ± 0.07	0	0.3 ± 0.27	3 / 10000
	F	24	5	0.402 ± 0.06	-4	0.1 ± 0.22	1 / 10000
2000 mg/m^3	M	24	5	0.409 ± 0.04	-4	0.3 ± 0.27	3 / 10000
	F	24	5	0.362 ± 0.02	-14	0.2 ± 0.27	2 / 10000
Saline	M	24	1	0.483 ± **	---	0.5 ± **	1 / 2000
	F	24	1	0.466 ± **	---	0.0 ± **	0 / 2000
Cyclophosphamide							
40 mg/kg	M	24	1	0.277 ± **	-43	10.5 ± **	*21 / 2000
	F	24	1	0.298 ± **	-36	10.0 ± **	*20 / 2000

*Statistically significant increase, $p \leq 0.05$

**Standard deviation not available due to data from single animal.

Table 2: Induction of Micronucleated Polychromatic Erythrocytes in Bone Marrow After Two Week Exposure of 344 Fischer Rats to FT Jet Fuel

Treatment	Sex	Animal Number	PCE/Total Erythrocytes	MN-PCE (Number/PCE scored)
Clean Air	M	101	0.402	0 / 2000
		102	0.489	1 / 2000
		103	0.359	0 / 2000
		104	0.514	0 / 2000
		105	0.373	1 / 2000
	F	201	0.397	1 / 2000
		202	0.437	0 / 2000
		203	0.355	1 / 2000
		204	0.452	1 / 2000
		205	0.461	0 / 2000
FT Jet Fuel 500 mg/m^3	M	106	0.378	0 / 2000
		107	0.372	0 / 2000
		108	0.388	0 / 2000
		109	0.300	0 / 2000
		110	0.359	0 / 2000
	F	206	0.380	1 / 2000
		207	0.453	0 / 2000
		208	0.369	1 / 2000
		209	0.319	0 / 2000
		210	0.366	0 / 2000
1000 mg/m^3	M	111	0.549	1 / 2000
		112	0.411	1 / 2000
		113	0.416	0 / 2000
		114	0.365	0 / 2000
		115	0.406	1 / 2000
	F	211	0.307	0 / 2000
		212	0.465	0 / 2000
		213	0.442	0 / 2000
		214	0.382	0 / 2000
		215	0.414	1 / 2000
2000 mg/m^3	M	116	0.422	0 / 2000
		117	0.468	0 / 2000
		118	0.370	1 / 2000
		119	0.415	1 / 2000
		120	0.371	1 / 2000
	F	216	0.352	0 / 2000
		217	0.329	0 / 2000
		218	0.389	1 / 2000
		219	0.368	0 / 2000
		220	0.372	1 / 2000
Saline	M	122	0.483	1 / 2000
	F	223	0.466	0 / 2000
Cyclophosphamide 40 mg/kg	M	129	0.277	21 / 2000
	F	226	0.298	20 / 2000

APPENDIX D. FINGERPRINT ANALYSIS OF FT JET FUEL BY GAS CHROMATOGRAPHY WITH MASS SPECTROMETRIC DETECTION

Mark A Sochaski, PhD.

Analytical Chemistry Services
The Hamner Institutes for Health Sciences

08/09/2010

INTRODUCTION

Production and use of a synthetic jet fuel (FT) for use in military aircrafts promises to reduce the exposure of military personnel to the potentially toxic aromatic hydrocarbons associated with the currently used JP-8 fuels. Although this new FT fuel has few, if any, aromatics in it, very little toxicity testing has been conducted in order to prove that it is a healthier alternative to working with the JP-8 fuels. The purpose of the exposures conducted at The Hamner was to assess the potential inhalation toxicology associated with a typical workplace exposure to FT jet fuel. Exposures were carried out at three concentrations and for two different time periods. In order to better understand any potential adverse biological observations the study may produce, a fingerprint analysis of the aerosol and vapor phase of the delivered test chemical mixture was requested by the sponsor. The goals associated with the fingerprint analysis were as follows:

1. Determination of the hydrocarbon fingerprint of FT jet fuel by GC/MS for future comparison to aerosolized FT jet fuel samples.

2. Qualitatively identify as many of the peaks present in the FT fuel mixture.

3. Collect and analyze FT jet fuel during an animal exposure with the intent of analyzing the aerosol phase and the gas phase independent of each other.

METHODS

Materials

- GC Column: Petrocol DH 150, Supelco, 150 m x 0.25 mm, 1.00 μm film, Cat #24155
- GC Liners: Siltek 4mm split with glass wool, Restek, Cat # 20782-213.5
- GC syringe: Hamilton gas tight syringe, Restek, Cat # 1701ASRN
- GC septa: 11 mm partial hole septa, Agilent, Part # 5181-3383
- Diesel range organics (DRO) test mix (Tenn/Miss), Restek, Cat # 31214
- Electrostatic precipitator, Aerosol Associates (Chapel Hill, NC)
- Dry Ice
- Ethanol
- Glass cold finger condenser, Prism Research Glass (Research Triangle Park, NC)
- FT jet fuel (POSF 5109)

Instrumental Conditions

- Gas Chromatograph: Agilent 6890 or Shimadzu GC-2010
- Mass Spectrometer: Agilent 5973N or Shimadzu GCMS QP-2010 plus
- Autosampler: Agilent 7683B (manual for Shimadzu)
- Chemstation software: Agilent version D.01.02.16
- Shimadzu software: GCMSsolution V.2.50 SU3
- NIST MS Database: NIST 2005 (Shimadzu)
- Total run time: 596 minutes
- Injection Size: 1 µL
- Split Ratio: 100:1
- Injector temperature: 250°C
- Transfer Line Temp: 280°C
- MS Quad Temp: 150°C
- MS Source Temp: 230°C
- MS Ionization Mode: Electron Impact
- Mass Scan Range: 35.0-350.0 amu

Table 1. Temperature program for Petrocol DH 150 column

Time (min)	**Initial Temperature** (°C)	**Final Temperature** (°C)	**Temperature Rate** (°C/min)	**Column Flow** (mL/min)	**Total Time** (min)
0.0	35	35	0	1	16
16.0	35	70	0.5	1	70
86	70	70	0	1	15
101	70	110	0.5	1	80
181	110	110	0	1	35
216	110	140	0.5	1	60
276	140	140	0	1	35
311	140	170	0.5	1	60
371	170	170	0	1	35
406	170	200	0.5	1	60
466	200	200	0	1	30
496	200	250	0.5	1	100
596	250	250	0	1	NA

Note: min = minutes

Compound Identification in FT Fuel

One milliliter of FT fuel was transferred directly to a silinated GC auto-sampler vial from the sample container (POSF 5109) provided by the U.S. Air Force (Wright Patterson Air Force Base, OH). Fuel transfer was done through the use of a 5 mL disposable Pyrex glass pipette which had been rinsed twice with FT prior to sample transfer. Samples were capped with Teflon lined crimp top caps and placed in the GC/MS auto-sampler for analysis. The diesel range organics (DRO) test mixture (quality control sample) was transferred to an identical auto-sampler vial using the same procedure. Analysis of the DRO test mixture was done prior to FT analysis and immediately following FT analysis to ensure consistent compound retention times were being observed (ensuring that the column itself was in good working condition) and to positively identify the elution times for the straight chain aliphatic compounds present in the fuel mixture.

Identification of the peaks was accomplished by matching with our mass spectral database. Matches were considered similar with QUAL values of 75 or better. These scores were calculated through a software algorithm (Agilent Chemstation) comparing the fragmentation ion pattern of the unknown to a known fragmentation pattern (National Institutes of Standards and Technology (NIST) database). The better match is indicated with a higher score (scores of 100 indicate a perfect match).

An effort was made to identify as many of the additional compounds present in the chromatogram as possible. Some of the assigned identifications were made through library matches that did not have scores above the arbitrarily set QUAL score acceptance criteria of 75, while the remaining identifications were made through manual mass spectral interpretation.

Analysis of Aerosol and Vapor Components

An electrostatic precipitator was generously donated by Dr. David Leith from the University of North Carolina at Chapel Hill, Department of Environmental Sciences. The selection of an electrostatic precipitator (ESP) for the collection of the aerosols was based on the published differences in the loss of sample (mass evaporated) from filter based aerosol collectors versus ESP collection methods[1-4]. At lower vapor concentrations, a significant amount of collected aerosol can be lost if filter sampling is used, as compared to ESP sampling, or that the more volatile components in the collected aerosol will be lost, thus producing a non-representative fingerprint of the actual aerosol components. The use of a cold trap in conjunction with the EPS was to ensure that even the most volatile components of the vapor phase were collected for analysis.

In order to collect the aerosol and vapor phase components of the jet fuel atmospheres, the electrostatic precipitator was hooked up, in series, with a cold finger glass trap using an ethanol/dry ice slurry bath. The precipitator removed any aerosol components present in the sample, while the cold finger trap removed all of the remaining volatile compounds. House vacuum (set to approximately 1.5 L/minute)[4] was used to pull sample from the exposure chambers through the electrostatic precipitator/cold trap set-up. For the high concentration exposure (2000 mg/m^3), a collection time of approximately 30 minutes was sufficient to collect

an acceptable (10-30 µL) amount of condensed aerosol. For the middle concentration exposure (1000 mg/m^3), a collection time of 75 minutes was used. For the low concentration exposure (500 mg/m^3), a collection time of >120 minutes was required. Following collection, samples were either directly injected onto a GC/MS (aerosol samples) or extracted with a minimal volume of carbon tetrachloride (vapor samples, comprised primarily of collected water vapor) prior to analysis.

Samples for the ten-day exposure were analyzed using a Shimadzu GCMS-2010 with QP-2010 plus MSD (Shimadzu, Kyoto, Japan). Aerosol and vapor fraction samples were analyzed on an Agilent 6890 GC with 5973 inert MSD (Agilent, Santa Clara, CA).

Confirmation of chamber fuel concentrations (total hydrocarbons) was conducted through the use of an infrared spectrophotometer (MIRAN 1A, Foxboro Co., South Norwalk, CT). A sample of the chamber atmosphere was continuously pulled through the heated cell of the IR spectrophotometer and the total signal obtained from the instrument was recorded. IR spectrophotometers were calibrated prior to use by analyzing a series of known jet fuel concentrations.

RESULTS

Compound Identification in FT Fuel

The known elution times of straight chain aliphatics can be used as markers for identifying the additional peaks present through the use of Figures 1-11. Identification of the peaks present in the FT fuel blend provided a considerable challenge as most of the highly branched hydrocarbons in the sample were not included in our mass spectral database. Table 2 summarizes the compounds with similarity match (or QUAL values) scores of 75 or better.

Despite the effort made to identify as many of the additional compounds present in the chromatogram as possible, there were still a large number of peaks which we were not able to assign to a specific compound due to the highly branched nature of many of the higher molecular weight hydrocarbons. Figures 1 through 11 illustrate 50 minute windows of the total chromatogram obtained during the analysis of the FT fuel blend on 02/28/2008 (during the ten-day exposure). All figures have the same X and Y axis scale. These figures include: A) The compounds found in Table 2, B) compounds identified through lower QUAL scores, and C) compounds determined through mass spectral interpretation.

Table 2. Identification of Analyte Peaks from FT Fuel

Compound	Retention time (minutes)	Qual Score
2-methyl-heptane	109.90	91
4-methyl-heptane	110.59	86
3-methyl-heptane	113.17	87
3-ethyl-hexane	113.74	78
n-Octane	124.37	91
2,2-dimethyl-heptane	132.83	78
2,4-dimethyl-heptane	134.49	91
2,6-dimethyl-heptane	137.11	78
Ethyl-cyclohexane	138.34	78
2,5-dimethyl-heptane	139.98	91
2,3-dimethyl-heptane	148.58	91
3,4-dimethyl-heptane	149.51	75
4-ethyl-heptane	150.38	91
4-methyl-octane	151.52	95
2-methyl-octane	151.93	94
3-ethyl-heptane	154.24	83
3-methyl-octane	154.74	91
n-Nonane	165.84	94
2-(2-methoxyethoxy)-ethanol	168.71	78
2,4,6-trimethyl-heptane	173.33	75
2,5-dimethyl-octane	176.05	95
3,5-dimethyl-octane	176.50	86
2,6-dimethyl-octane	179.55	90
3,3-dimethyl-octane	180.41	78
2,3-dimethyl-octane	187.57	91
4-ethyl-octane	187.87	93
5-methyl-nonane	189.56	95
4-methyl-nonane	190.33	91
2-methyl-nonane	191.398	96
3-ethyl-octane	192.93	90
3-methyl-nonane	194.27	91
1,3,5-trimethyl-benzene	199.58	76
n-Decane	207.16	97
5-ethyl-2-methyl-octane	214.628	94
2,5-dimethyl-nonane	217.653	94
5-ethyl-2-methyl-octane	233.34	80
2,3-dimethyl-nonane	234.398	94
5-methyl-decane	235.87	93
4-methyl-decane	237.38	95
2-methyl-decane	239.05	95
3-methyl-decane	242.10	95
n-Undecane	254.99	96
4-ethyl-decane	260.080	87
3,5-dimethyl-decane	261.748	78
2,6-dimethyl-decane	262.978	95
3,6-dimethyl-decane	264.916	97
3,7-dimethyl-decane	266.981	94
5-propyl-nonane	267.578	78
5-ethyl-decane	273.61	93

Compound	Retention time (minutes)	Qual Score
4-ethyl-decane	275.69	76
6-methyl-undecane	277.395	90
5-methyl-undecane	277.94	93
4-methyl-undecane	279.498	94
2-methyl-undecane	281.238	91
3-methyl-undecane	284.03	90
n-Dodecane	296.581	96
2,5-dimethyl-undecane	303.677	95
3,8-dimethyl-undecane	310.874	80
2,9-dimethyl-undecane	313.461	83
4-ethyl-undecane	320.013	90
6-methyl-dodecane	321.922	81
5-methyl-dodecane	322.904	86
4-methyl-dodecane	325.085	97
2-methyl-dodecane	327.233	97
3-ethyl-dodecane	328.118	90
3-methyl-dodecane	330.397	90
n-Tridecane	342.914	98
7-methyl-tridecane	362.793	81
6-methyl-tridecane	363.134	90
5-methyl-tridecane	364.184	96
4-methyl-tridecane	366.013	96
2-methyl-tridecane	367.899	94
3-methyl-tridecane	370.440	96
n-Tetradecane	381.026	98
7-methyl-tetradecane	400.692	85
6-methyl-tetradecane	401.378	85
5-methyl-tetradecane	402.731	98
4-methyl-tetradecane	405.160	98
2-methyl-tetradecane	407.462	98
3-methyl-tetradecane	410.671	98
n-Pentadecane	422.591	98
7-methyl-pentadecane	440.787	90
6-methyl-pentadecane	441.511	75
5-methyl-pentadecane	442.830	75
4-methyl-pentadecane	444.899	94
2-methyl-pentadecane	446.766	91
3-methyl-pentadecane	449.379	75
n-Hexadecane	458.856	98

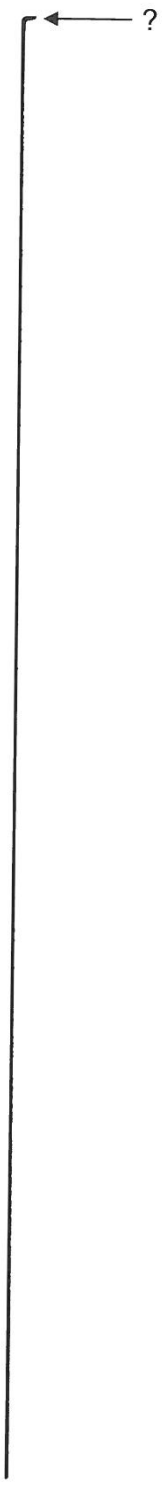

Figure 1. FT Fuel Analysis, 0-50 Minute Window

Figure 2. FT Fuel Analysis, 50-100 Minute Window

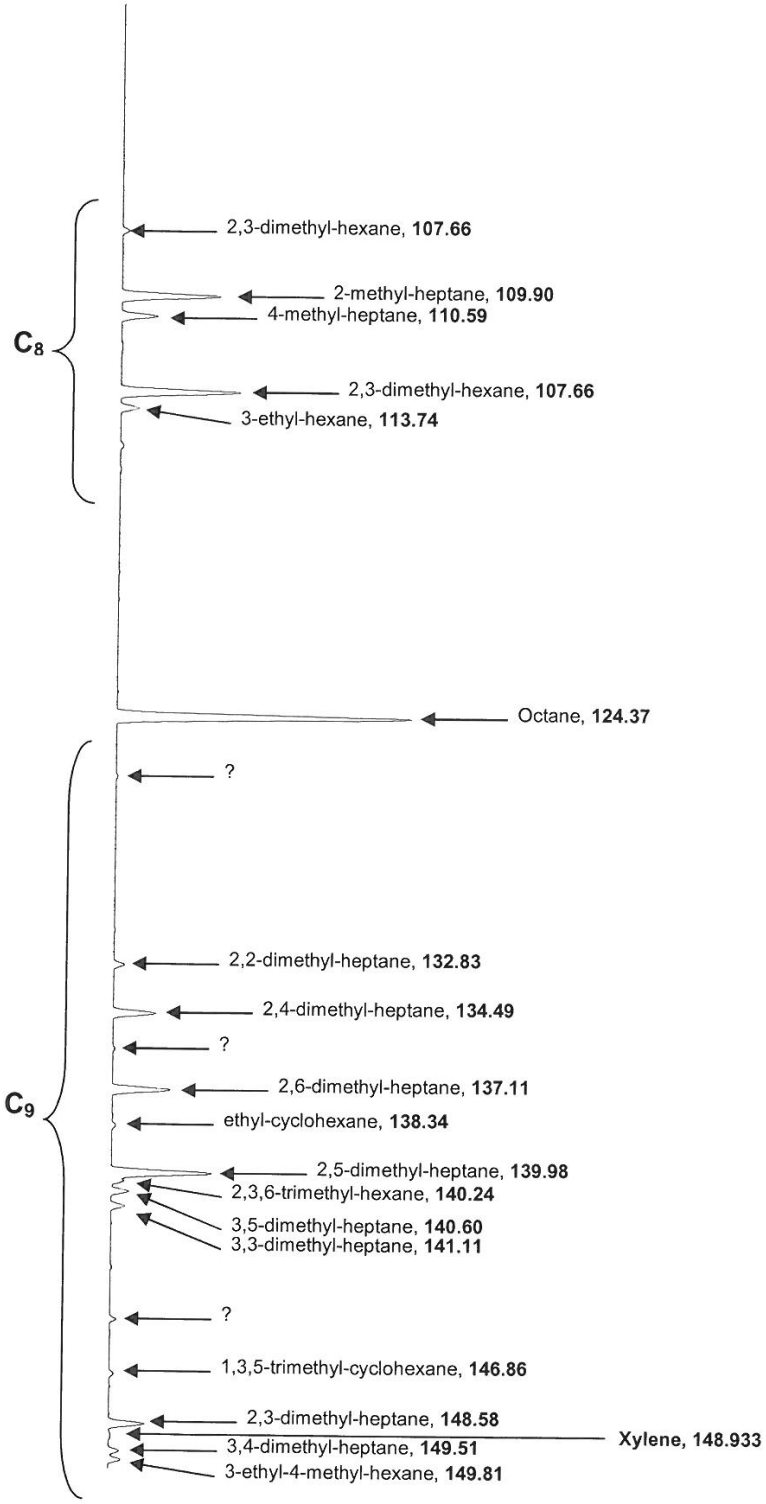

Figure 3. FT Fuel Analysis, 100-150 Minute Window

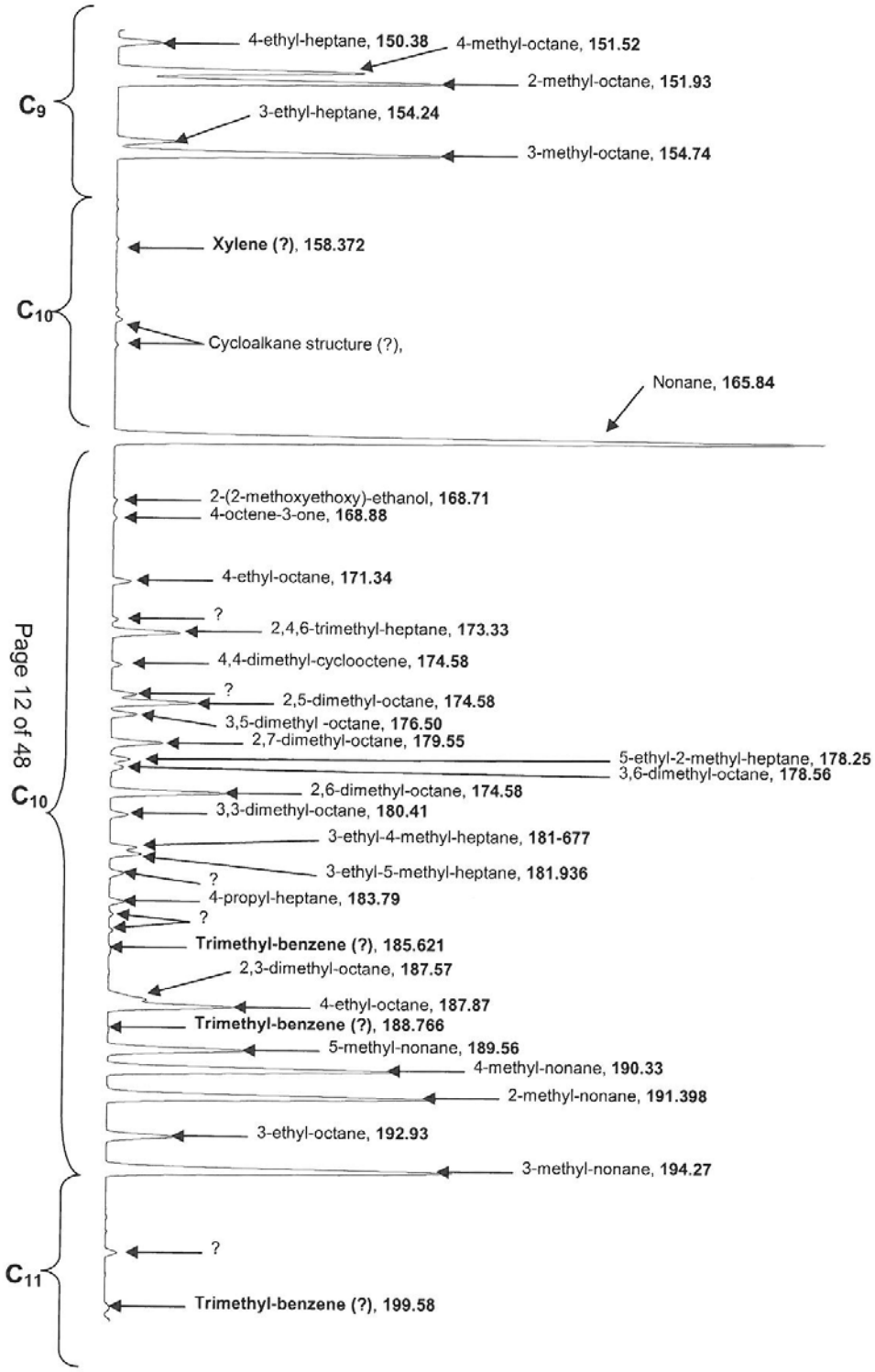

Figure 4. FT Fuel Analysis, 150-200 Minute Window

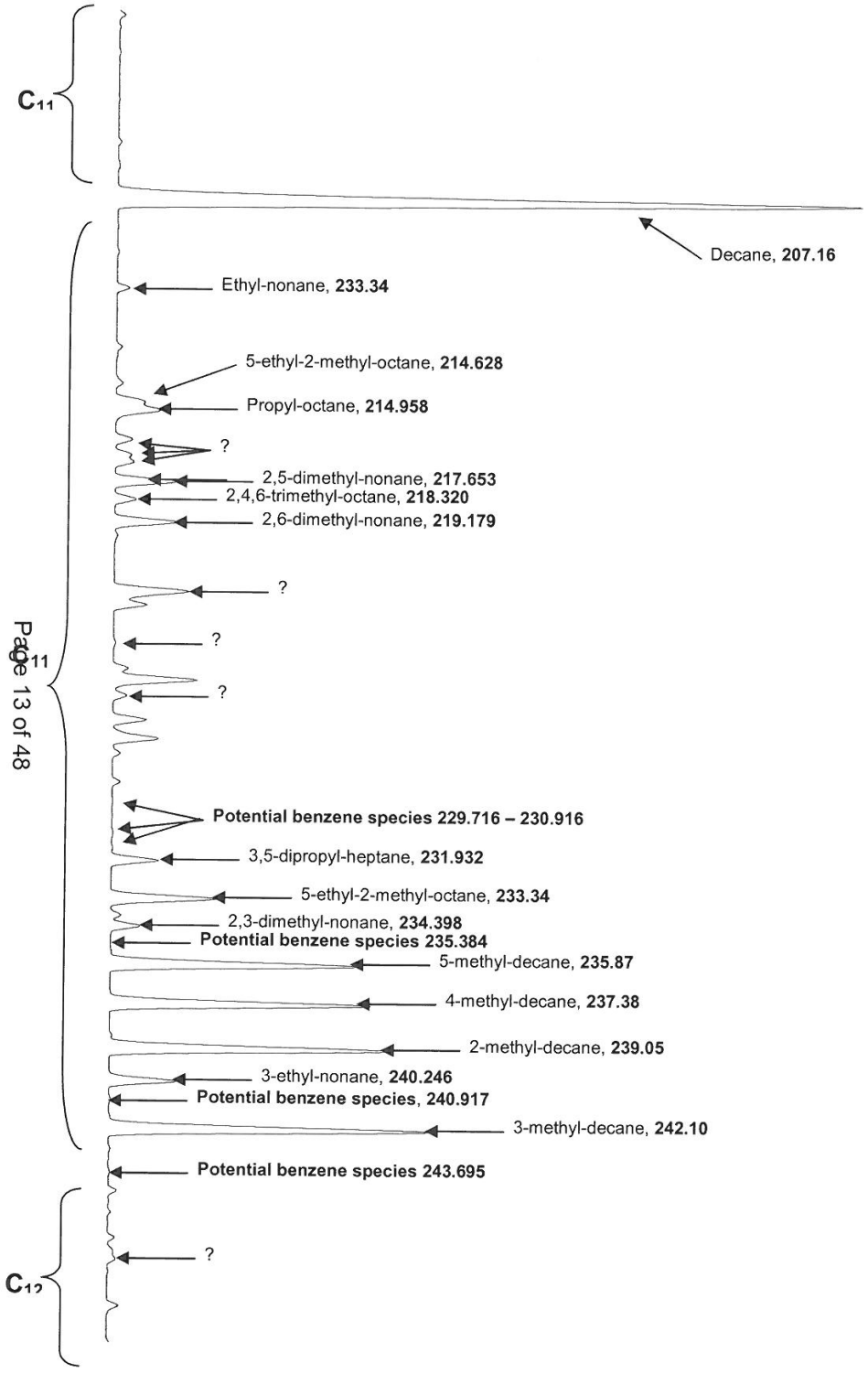

Figure 5. FT Fuel Analysis, 200-250 Minute Window

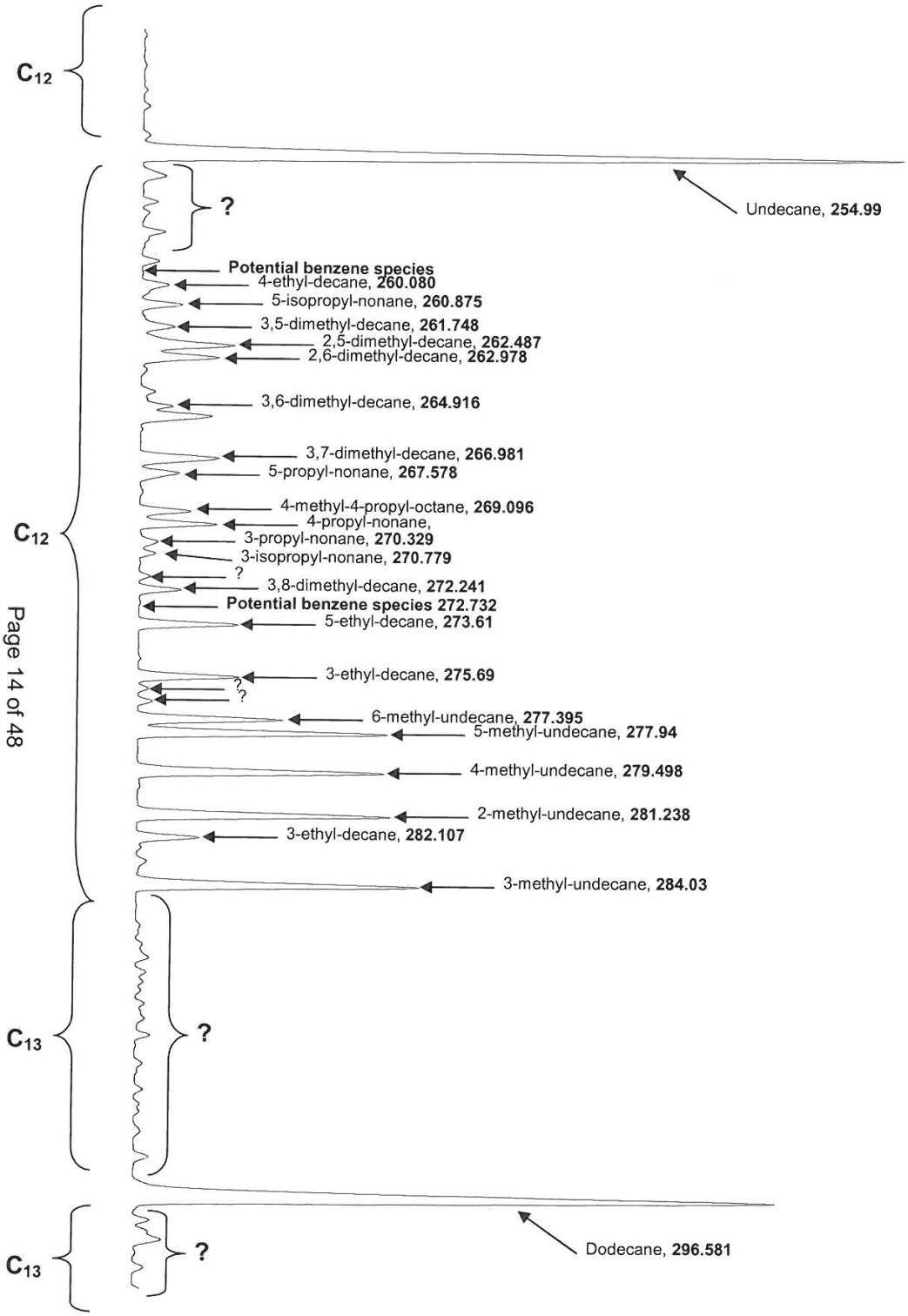

Figure 6. FT Fuel Analysis, 250-300 Minute Window

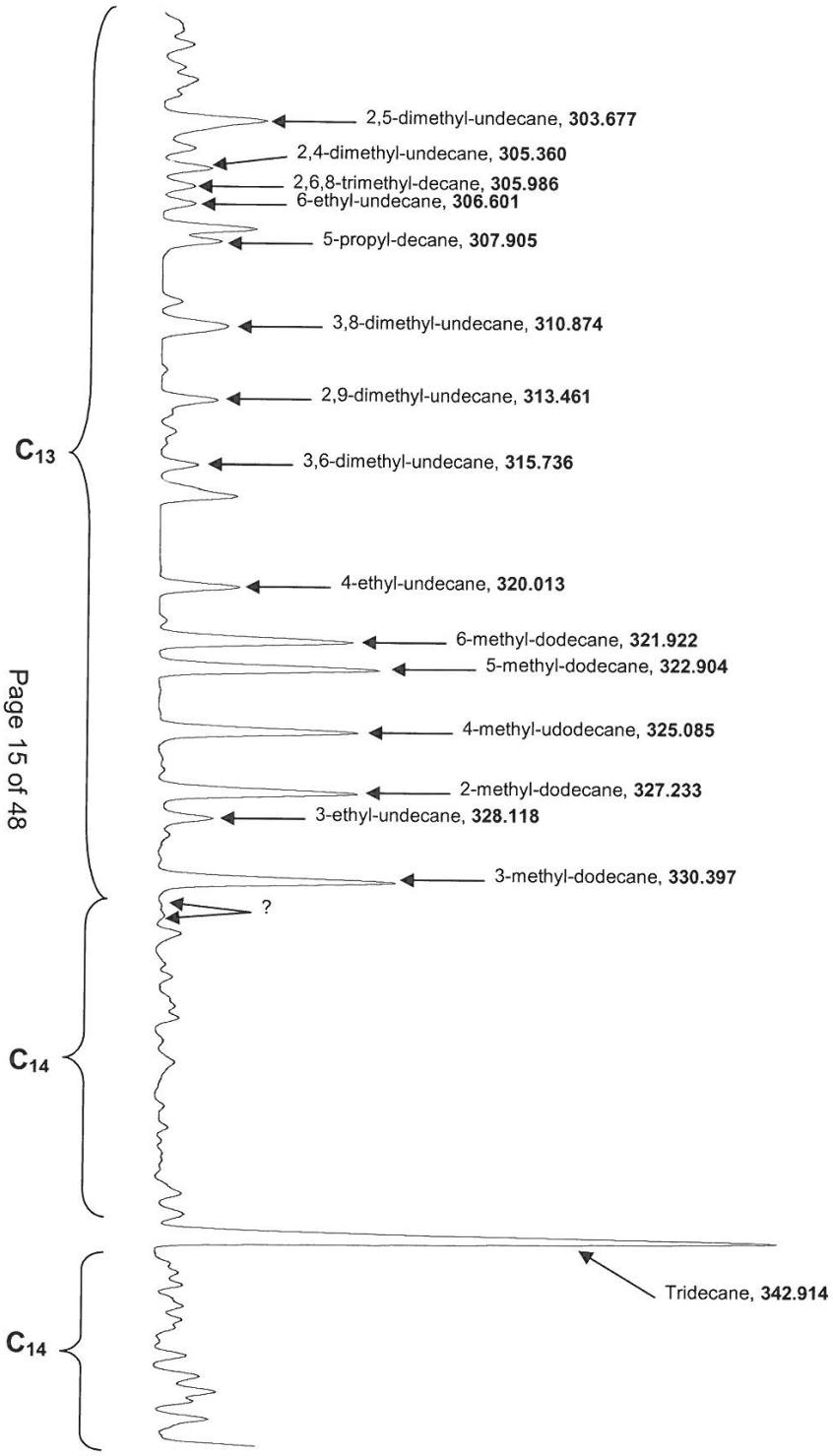

Figure 7. FT Fuel Analysis, 300-350 Minute Window

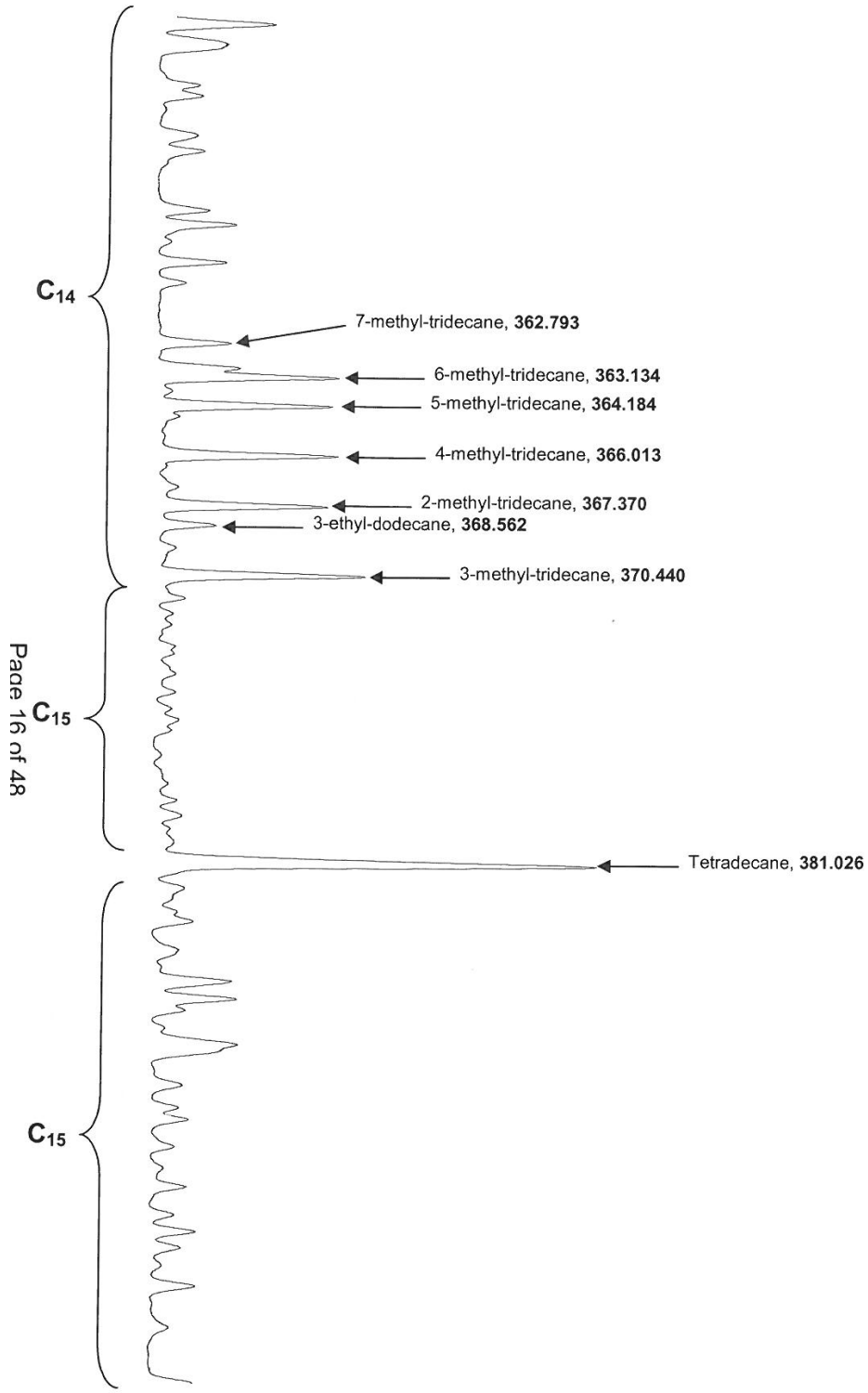

Figure 8. FT Fuel Analysis, 350-400 Minute Window

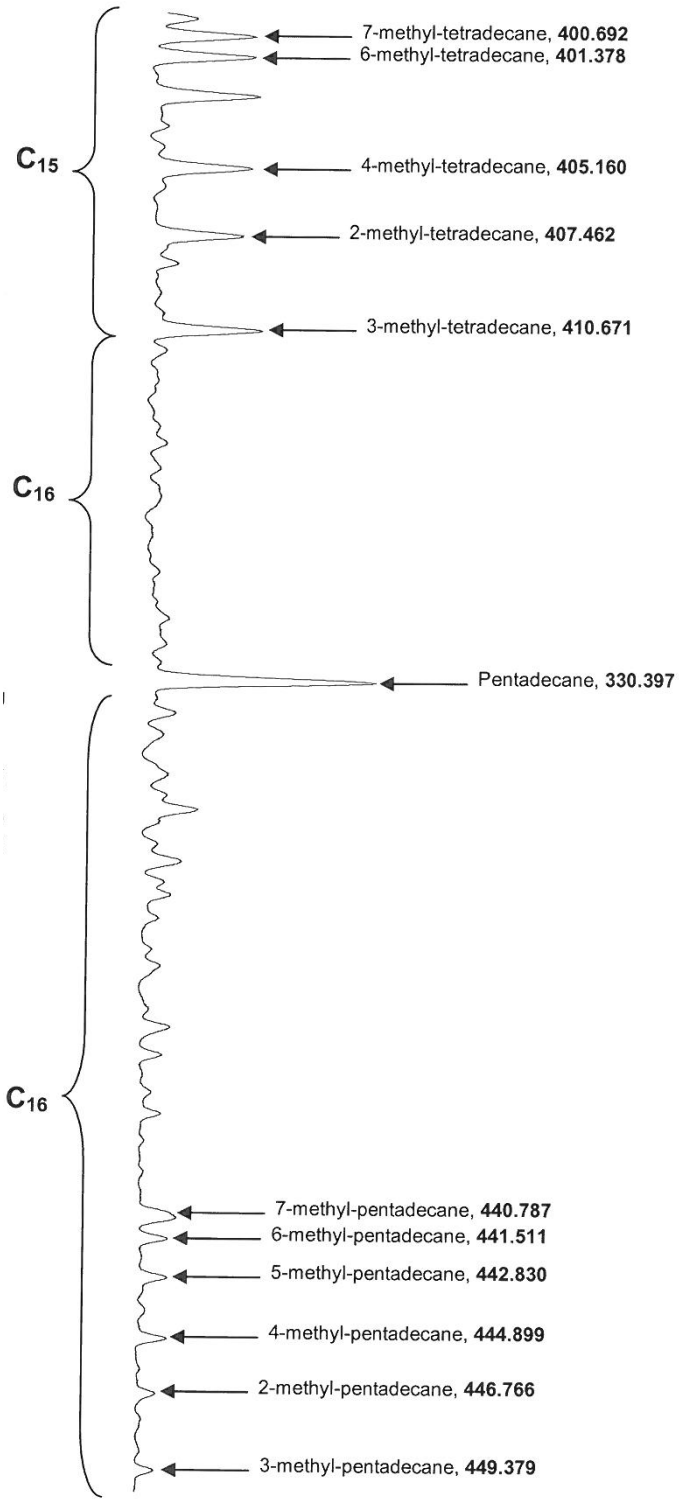

Figure 9. FT Fuel Analysis, 400-450 Minute Window

Figure 10. FT Fuel Analysis, 450-500 Minute Window

Figure 11. FT Fuel Analysis, 500-550 Minute Window

During the analysis of the peaks present in the fuel mix, several peaks containing mass spectra with a 91 amu mass fragment (possibly originating from methyl substituted benzene molecules)

were observed. It should be noted that these "aromatic" peaks were extremely small and assigned their identifications based on very weak mass spectra signals. A conclusive qualitative analysis for aromatic elution times was not carried out to verify these peak identities. These potential aromatic peaks are labeled in Figures 1 through 11 in bold type. A complete chromatogram for the FT fuel analysis is shown in Figure 12 (pre-exposure analysis, ten-day exposure). Figure 13 illustrates the profile of the DRO test mix for determining elution times of the major constituents in the FT Jet fuel mixture. The elution times of the straight chain aliphatic hydrocarbons agree closely with the mass spectra library identified straight chains labeled in Figure 12; the difference in elution time is attributable to the difference in matrix complexity between the two samples. The DRO test mix contains only long straight chain aliphatic hydrocarbons.

Analysis of Aerosol and Vapor Components

Actual chamber concentrations for the days of sample collection are located in the appropriate figure legends for the aerosol and vapor phase analysis figures (Figures 14 through 16).

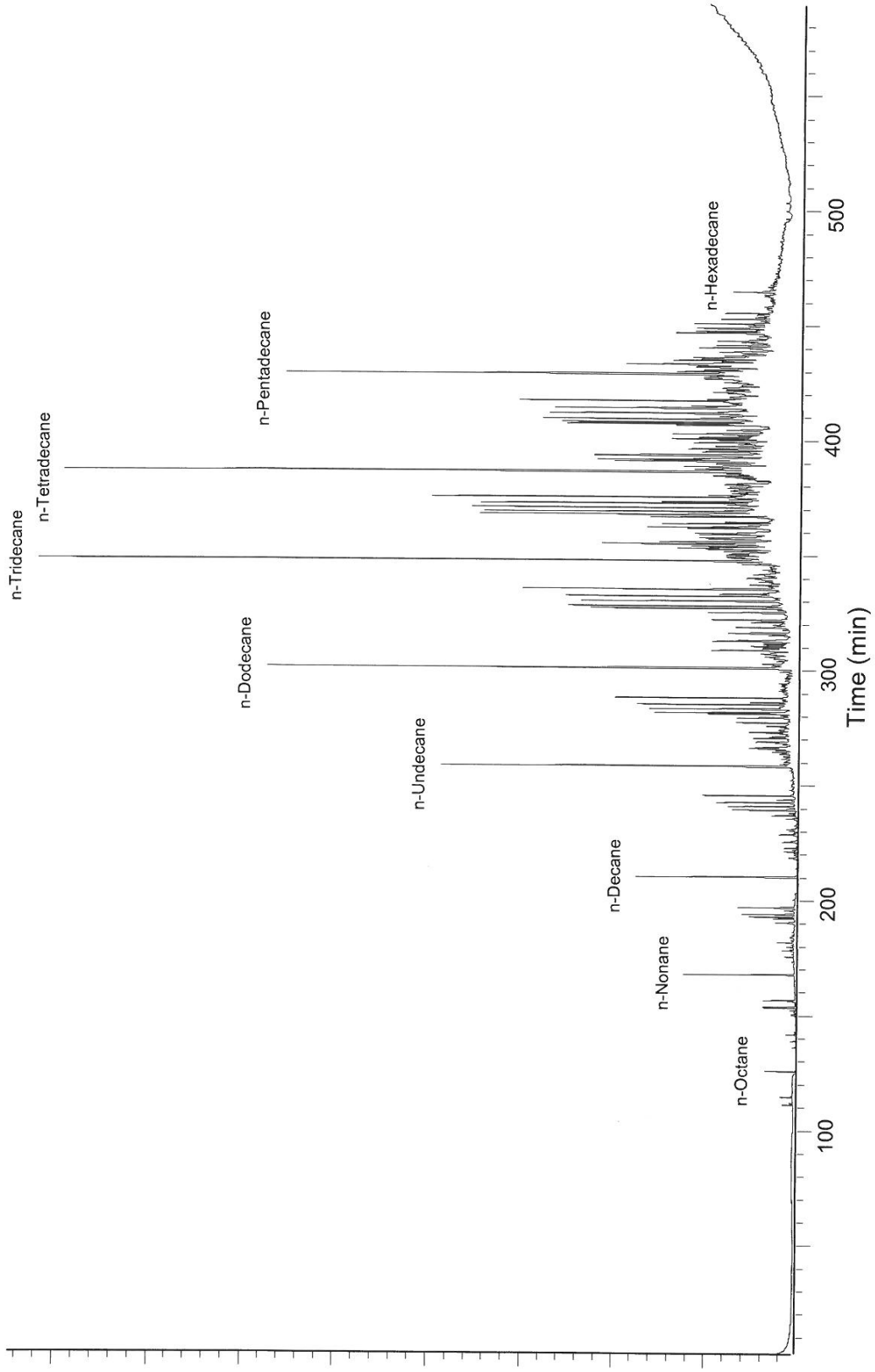

Figure 12. GC/MS Pre-Study Analysis of FT Jet Fuel

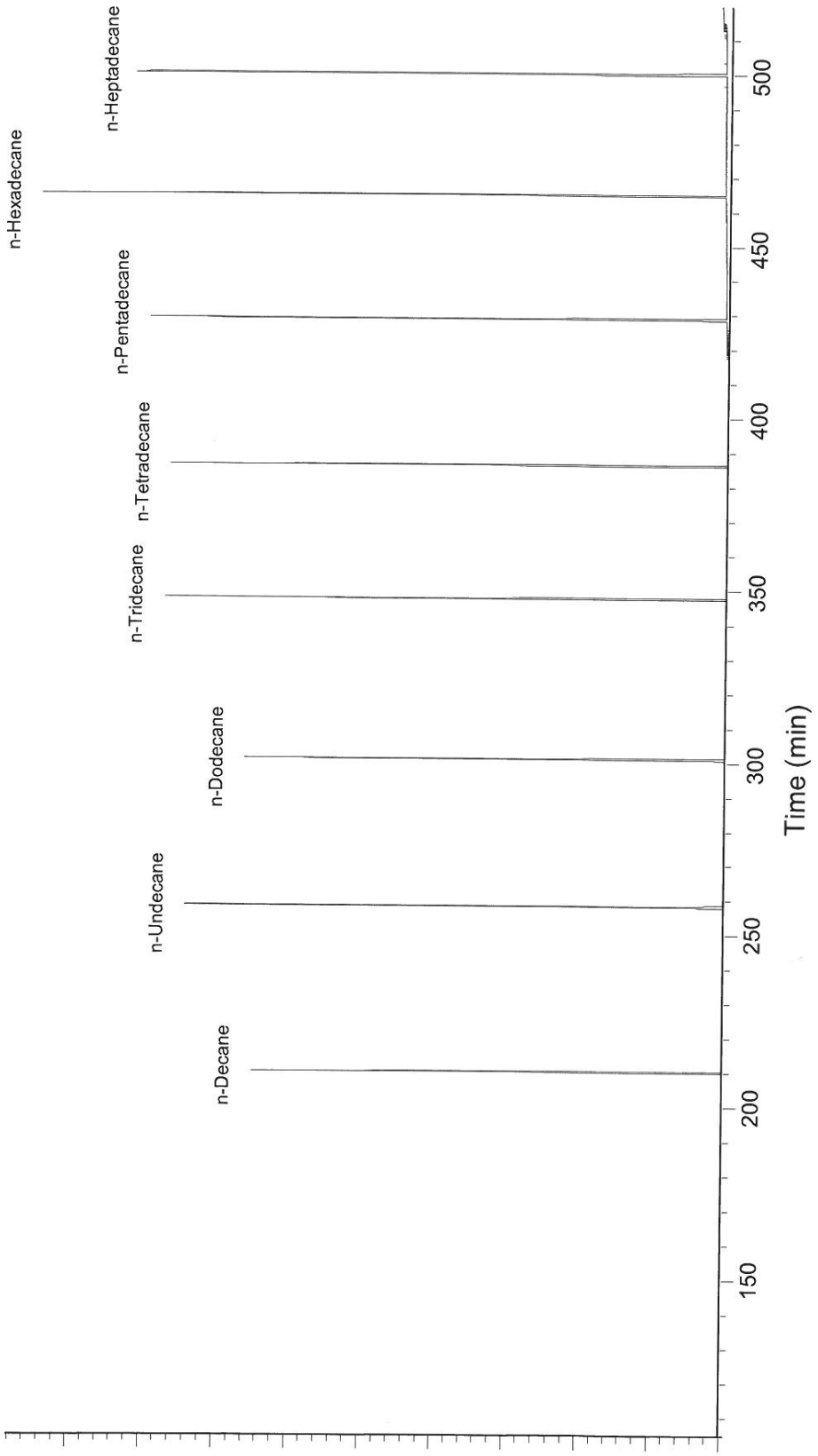

Figure 13. GC/MS Analysis of DRO Test Mix

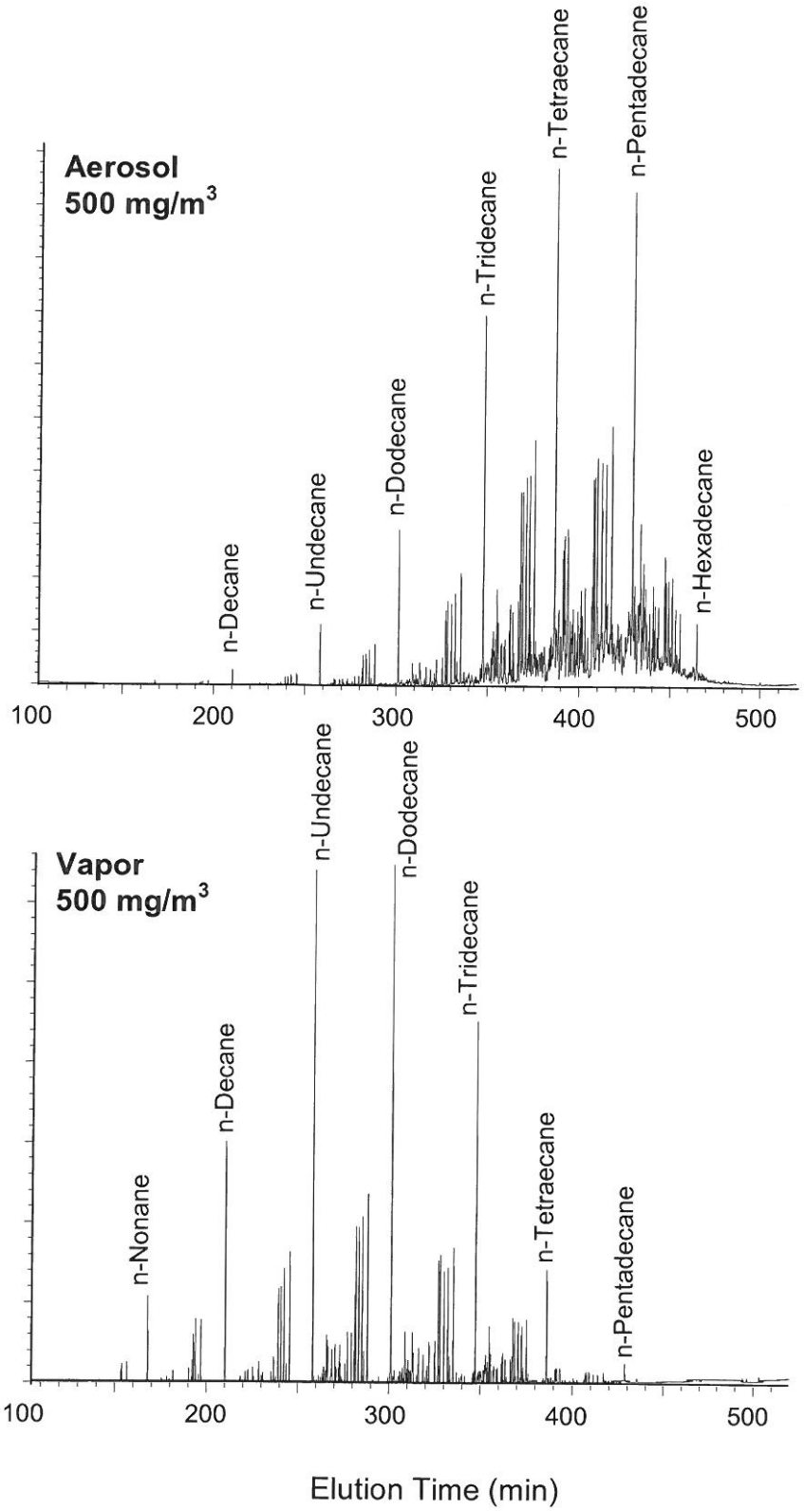

Figure 14. Aerosol/Vapor Fraction, Ten-Day Exposure, 500 mg/m^3
Actual chamber concentration = 500.0 mg/m^3

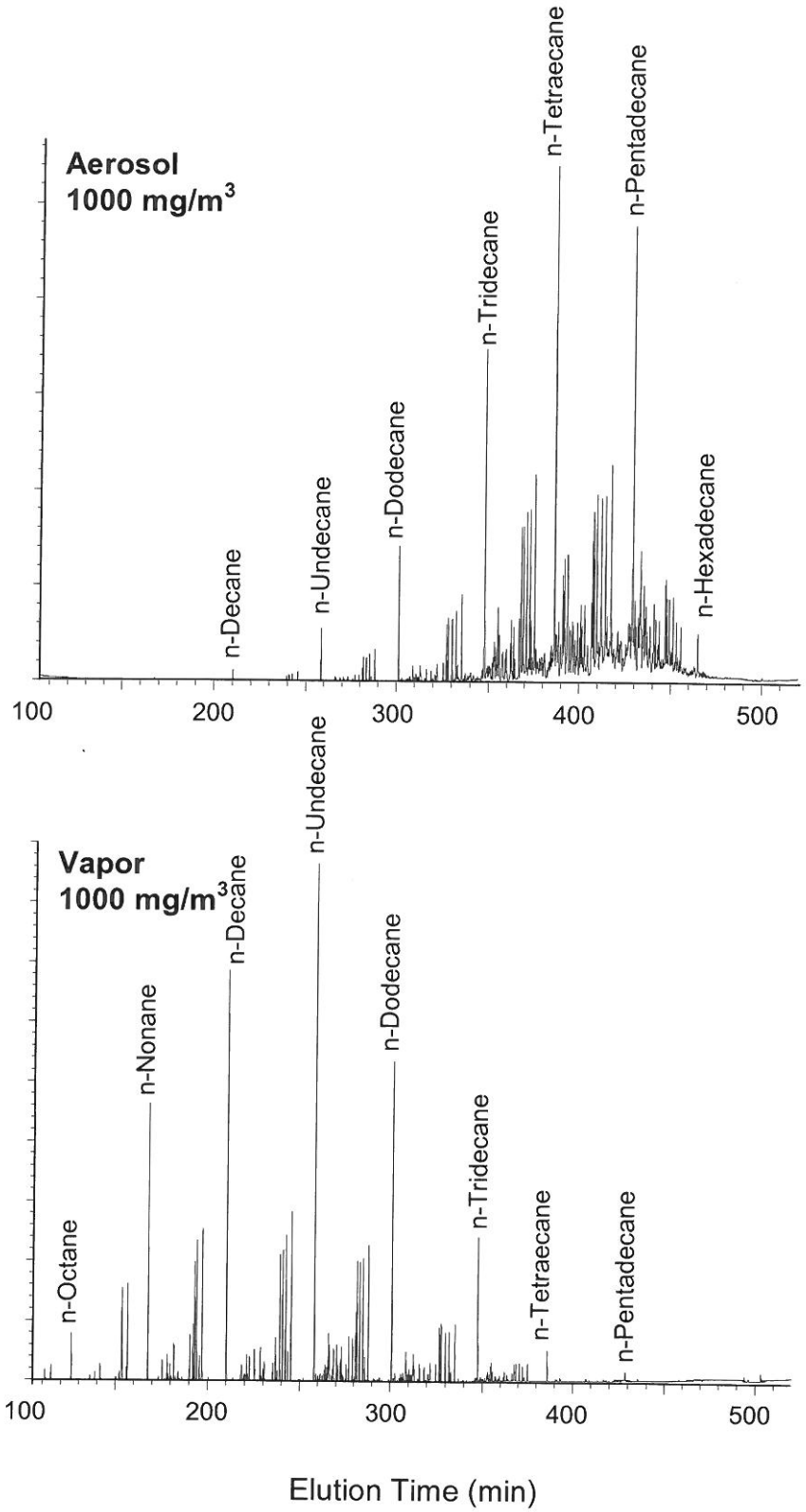

Figure 15. Aerosol/Vapor Fraction, Ten-Day Exposure, 1000 mg/m^3
Actual chamber concentration = 1012.5 mg/m^3

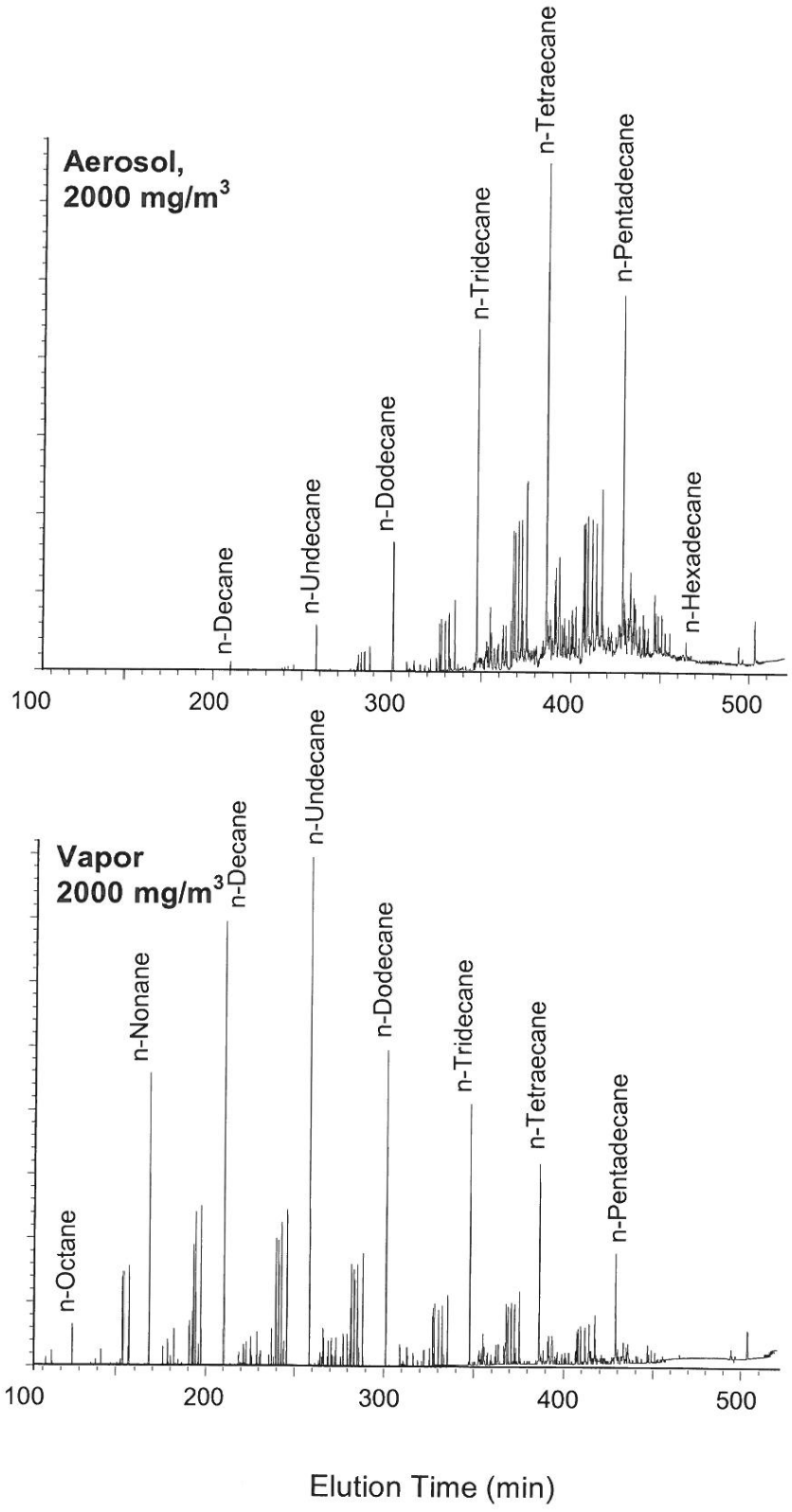

Figure 16. Aerosol/Vapor Fraction, Ten-Day Exposure, 2000 mg/m^3
Actual chamber concentration = 1972.1 mg/m^3

CONCLUSIONS

The following conclusions were made based on the qualitative analysis of the GC/MS analysis of the collected samples:

1. In all cases, there was an apparent shift in the distribution of the chemical species present in the jet fuel samples. This shift demonstrated an increased presence of high molecular weight compounds in the aerosol phase compared to an increased presence of low molecular weight compounds in the vapor phase. This trend was observed at all three exposure concentrations.

2. There did not appear to be an appreciable difference in the distribution of compounds when comparing the different aerosol fractions from each concentration group.

3. In the vapor phase, there appeared to be more total compounds present in the high concentration exposure samples compared to the low concentration exposure samples.

4. A majority of the compounds (accounting for >90 percent of the total peak area in the sample) found in the low concentration vapor samples were those found between n-undecane and n-tetradecane. The high concentration vapor samples, meanwhile, appeared to contain a much larger range of molecular weight compounds (i.e., n-octane through n-pentadecane was observed).

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3) Cardello N., Volckens J., Tolocka M. P., Wiener R., Buckley T. J., Technical note: Performance of a personal electrostatic precipitator particle sampler. (2002) Aerosol Sci. Tech., 36: 162-165

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Approval Signatures

This report is an accurate and complete representation of the data for this study (Protocols 08002 and 08013)

Signature: _____ 08/09/2010

Mark Sochaski, Date
The Hamner Institutes for Health Sciences
Analytical Chemistry Services, Manager

LIST OF ABBREVIATIONS

AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care International
ACGIH	American Conference of Industrial Hygienists
AFRL/RZPF	Air Force Research Laboratory Fuels Branch
APS	aerodynamic particle sizer
CP	cyclophosphamide
CV	coefficient of variation
DRO	diesel range organics
EC	total erythrocytes
EPL	Experimental Pathology Laboratories, Inc.
ESP	electrostatic precipitator
FMI	Fluid Metering, Inc.
FT	Fischer Tropsch
GC	gas chromatograph
GC/MS	gas chromatography/mass spectroscopy
GLP	Good Laboratory Practices
GSD	geometric standard deviation
H&E	hematoxylin and eosin
HEPA	high efficiency particulate air
IP	intraperitoneally
IR	infrared
MMAD	mass median aerodynamic diameter
MN	micronucleus
MPCEs	micronucleated polychromatic erythrocytes
NCEs	normochromatic erythrocytes
NIST	National Institutes of Standards and Technology
NOEL	no observable effect level
NRC	National Research Council
OECD	Organisation for Economic Co-operation and Development Guideline
PCEs	polychromatic erythrocytes
PEL	permissible exposure limit
SD	standard deviation
TP	total port
U.S. EPA	U.S. Environmental Protection Agency
WP	within port
WPAFB	Wright Patterson Air Force Base